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(FILE 'HOME' ENTERED AT 14:14:13 ON 24 JUL 2003)

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L2         38365 S TRANSGENIC
L3             0 S L1 (S) L2
L4         23829 S KNOCKOUT
L5             0 S L1 (S) L4
L6             0 S L1 (L) L4
L7             1 S L1 (L) L2
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L10           24 S L6
L11           31 S L7
L12           32 DUP REM L8 L9 L10 L11 (33 DUPLICATES REMOVED)
L13          548 S ERK5 OR BMK1
L14            6 S L2 (S) L13
L15      1924901 S REVIEW
L16            9 S L1 (S) L15
L17            5 DUP REM L16 (4 DUPLICATES REMOVED)
L18            1 S L17 NOT PY>1999
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13	11496	knockout	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/07/24 13:58
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1	29	erk5	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/07/24 14:07
31	2	erk5.clm.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/07/24 14:02
37	15	erk5 and transgenic	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/07/24 14:07

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Please provide the following article:

- TITLE: Investigation of BMK1/ERK5 Mitogen Activated Protein (MAP) kinase signaling using a conditional knockout mouse model
AUTHOR: Lee J D (Reprint); Hayashi M
CORPORATE SOURCE: Scripps Res Inst, La Jolla, CA 92037 USA
COUNTRY OF AUTHOR: USA
SOURCE: FASEB JOURNAL, (14 MAR 2003) Vol. 17, No. 4, Part 1, Supp. [S], pp. A602-A602.

- TITLE: Investigation of BMK1/ERK5 Mitogen Activated Protein (MAP) kinase signaling using a conditional knockout mouse model
AUTHOR: Lee J D (Reprint); Hayashi M
CORPORATE SOURCE: Scripps Res Inst, La Jolla, CA 92037 USA
COUNTRY OF AUTHOR: USA
SOURCE: FASEB JOURNAL, (14 MAR 2003) Vol. 17, No. 4, Part 1, Supp. [S], pp. A602-A602.
Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.

[redacted]

- ACCESSION NUMBER: 1999043491 EMBASE
TITLE: Mitogen-activated protein kinase: Conservation of a three-kinase module from yeast to human.
AUTHOR: Widmann C.; Gibson S.; Jarpe M.B.; Johnson G.L.
CORPORATE SOURCE: C. Widmann, Inst. Biol. Cellulaire Morphologie, University of Lausanne, Ch. du Bugnon 9, 1005 Lausanne, Switzerland
SOURCE: Physiological Reviews, (1999) 79/1 (143-180).
Refs: 409

10/2039458

- TITLE: Mitogen-activated protein kinase signal transduction in skeletal muscle: Effects of exercise and muscle contraction.
AUTHOR(S): Widegren, U.; Ryder, J. W.; Zierath, J. R. (1)
CORPORATE SOURCE: (1) Division of Integrative Physiology, Karolinska Institutet, S-171 77, Stockholm Sweden
SOURCE: Acta Physiologica Scandinavica, (July, 2001) Vol. 172, No. 3, pp. 227-238. print.

Thanks.

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Mitogen-activated protein kinas signal transduction in skel tal muscl : effects of x rcise and muscle contraction

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ABSTRACT

Exercise has numerous growth and metabolic effects in skeletal muscle, including changes in glycogen metabolism, glucose and amino acid uptake, protein synthesis and gene transcription. However, the mechanism(s) by which exercise regulates intracellular signal transduction to the transcriptional machinery in the nucleus, thus modulating gene expression, is largely unknown. This review will provide insight on potential intracellular signalling mechanisms by which muscle contraction/exercise leads to changes in gene expression. Mitogen-activated protein kinase (MAPK) cascades are associated with increased transcriptional activity. The MAPK family members can be separated into distinct parallel pathways including the extracellular signal-regulated kinase (ERK) 1/2, the stress-activated protein kinase cascades (SAPK1/JNK and SAPK2/p38) and the extracellular signal-regulated kinase 5 (ERK5). Acute exercise elicits signal transduction via MAPK cascades in direct response to muscle contraction. Thus, MAPK pathways appear to be potential physiological mechanisms involved in the exercise-induced regulation of gene expression in skeletal muscle.

Keywords exercise, extracellular signal-regulated kinase, gene expression, mitogen-activated protein kinase, muscle contraction, stress-activated protein kinase.

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The effects of exercise on metabolic and morphological responses/adaptations are highly specific to exercise type, frequency, and duration. Although exercise-training-induced adaptations in skeletal muscle are the result of cumulative effects of repeated bouts of exercise, the initial signalling responses that lead to long-term adaptations presumably occur in relation to each single training session. Exercise is a very complex physiological stimulus that challenges multiple biochemical and biophysical aspects of cellular function and therefore requires an appropriate control network. However, at present, the molecular signalling mechanisms linking muscle contraction or other exercise-associated stimuli to intracellular responses in skeletal muscle are poorly understood.

Among the observed benefits of exercise training are increased whole-body glucose disposal and improved insulin sensitivity (Houmard *et al.* 1991, Hughes *et al.* 1993). These effects appear to be mediated via increased expression of the glucose transporter protein 4 (GLUT4) (Houmard *et al.* 1991, Hughes *et al.* 1993, Ren *et al.* 1994, Ploug *et al.* 1998, Chibalin *et al.* 2000), as well as adaptive responses in the expression and function of key insulin-signalling molecules (Houmard *et al.* 1999, Chibalin *et al.* 2000). The cellular signalling mechanism that mediates exercise-induced adaptations in skeletal muscle may involve the mitogen-activated protein kinase (MAPK) signalling cascades as MAPK activation has been implicated as an important mechanism governing cellular proliferation and differentiation (Brunet & Pouyssegur 1997, Karin 1995).

Mitogen-activated protein kinase cascades

In mammalian cells, numerous intracellular signalling cascades are known to be critical intermediates in the regulation of various biological functions (Widmann *et al.* 1999). Cellular stress can elicit signal transduction through protein phosphorylation on serine, threonine, and tyrosine residues, and this constitutes a major regulatory mechanism that utilizes second messenger systems coupled to cell surface receptors (Cooper &

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Hunter 1983, Ray & Sturgill 1988, Anderson *et al.* 1990). The MAPK are ubiquitous proteins that transduce extracellular signals leading to a variety of cellular responses including proliferation, differentiation, apoptosis and adaptation to environmental stress (Pages *et al.* 1993, Kyriakis *et al.* 1994, Ichijo *et al.* 1997, Leppa *et al.* 1998). This can have important implications in the exercise response as exercise can be considered a physiological form of cellular stress. The parallel MAPK pathways include extracellular signal-regulated kinases (ERK) 1/2, the two stress-activated protein kinase cascades SAPK1/JNK and SAPK2/p38, and the extracellular signal-regulated kinase 5 (ERK5), also named big MAPK1 (BMK1). A defining characteristic of a MAPK is the requirement of dual phosphorylation of a Thr–X–Tyr motif for full enzyme activation, where X can be different amino acids among different MAPKs. While MAPK signals lead to diverse downstream events, the MAPK-signalling cascades *per se* are somewhat conserved, in that the pathways involve similar three kinase modules (Brunet & Pouyssegur 1997, Widmann *et al.* 1999). For example, ERK is activated by phosphorylation by a MAPK/ERK kinase (MEK), which is similarly activated by phosphorylation of an upstream MEK kinase (MEKK) (Fig. 1).

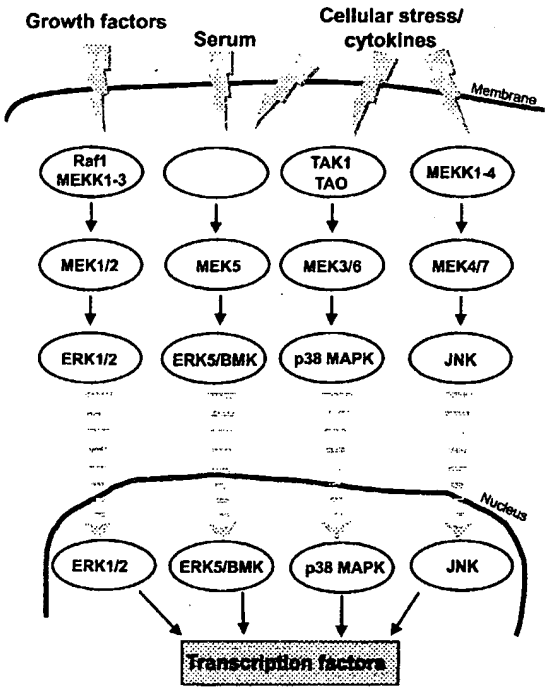

Figure 1 Proposed hierarchy of the MAPK module. Schematic of the ERK, p38 and JNK MAPK signalling pathways derived from investigations using cultured cell systems.

Many factors including growth factors, cytokines, hypoxia, changes in intracellular calcium and mechanical stress have also been shown to stimulate MAPK signal cascades (Table 1). Several of these factors are associated with exercise. Thus, MAPK signal transduction cascades are candidate systems that may convert the mechanical/biochemical stimuli elicited by muscle contraction into appropriate intracellular responses. MAPK signalling pathways have been implicated in the regulation of gene transcription in numerous cell types (Kyriakis *et al.* 1994, Cavigelli *et al.* 1995, Gupta *et al.* 1995, Xing *et al.* 1996). Constitutes of the MAPK signalling pathways are expressed in all muscle cell types (skeletal, cardiac and smooth) (Yamazaki *et al.* 1995, Goodyear *et al.* 1996, Force & Bonventre 1998). However, the majority of studies assessing the role of the MAPK signalling cascades have been performed in cardiac myocytes and vascular smooth muscle. In cultured cardiac myocytes, MAPK signalling is stimulated by mechanical stretch (Yamazaki *et al.* 1993, Yamazaki *et al.* 1995) and this may play a role in stretch-induced hypertrophy. Thus, relatively little is known of the regulation of these signalling cascades in skeletal muscle.

Table 1 Exercise-induced factors that may stimulate MAPK signal transduction cascades

Activator	MAP kinase	Cell system	Reference
Growth factors	MEK, ERK	Cardiomyocytes	Wang *et al.* (1998)
Growth hormone	Ras/MEK/ERK	3T3-F442A cells	Hodge *et al.* (1998)
Cytokine (IL-6)	ERK pathway	Multiple myeloma cells	Ogata *et al.* (1997)
TNF-α	JNK	HepG2, porcine hepatocytes	Kyriakis *et al.* (1994)
Integrins	ERK pathway	Murine NIH3T3 fibroblasts	Schlaepfer *et al.* (1994)
Changes in osmolarity	p38 MAPK	Murine pre-B cells transfected with human CD14	Han *et al.* (1994)
Stretch	JNK	Cardiac myocytes	Komuro *et al.* (1996)
Hypoxia	MEK/ERK/$p90^{rsk}$	Cardiac myocytes	Seko *et al.* (1996)
Heat shock	JNK	NIH3T3, HT-29	Kyriakis *et al.* (1994)
Ca^{2+}	ERK/RSK2/CREB	PC12 cells, hippo-campal neurons	Impey *et al.* (1998)
Hydroxyl radicals	Ras/Raf/ERK	Cardiac myocytes	Aikawa *et al.* (1997)

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Extracellular signal-regulated kinase

The ERK1/2 signalling is the most characterized MAPK pathway in mammalian cells and is activated through many different receptor types such as the epidermal growth factor receptor, neural growth factor receptor or insulin receptor (Boulton *et al.* 1991, Seger *et al.* 1992). Additionally, activation of ERK1/2 occurs upon stimulation of G-protein-coupled receptors (Crespo *et al.* 1994), growth factors (Wang *et al.* 1998), hormones (Hodge *et al.* 1998), cytokines (Winston & Hunter 1996, Ogata *et al.* 1997), or integrins (Schlaepfer *et al.* 1994). Classical receptor tyrosine kinase activation of ERK1/2 is initiated by receptor autophosphorylation on tyrosine residues, which promotes the formation of a Shc–Grb2 complex, containing SH2- and SH3 domains (Skolnik *et al.* 1993). This complex associates with the guanine nucleotide exchange factor Sos at the plasma membrane and facilitates a conversion of the inactive GDP–Ras to the active GTP–Ras (Downward 1996). This leads to an activation of Ras and Raf (MEKK), which phosphorylates the MAPK kinase (MEK1/2), the upstream kinase of ERK1/2 (Zheng & Guan 1993). In addition, ERK activation can occur through mechanisms involving activation of protein kinase C (PKC) (Schonwasser *et al.* 1998). Several cytokine receptors also activate ERK1/2 through the activation of JAK (Janus Kinases) (Stancato *et al.* 1997). Downstream cytosolic targets for ERK1/2 include MAPK activated protein kinase (MAPKAPK) 1 (also called p90 ribosomal S6 kinase; p90rsk) (Zhao *et al.* 1996), and mitogen- and stress-activated protein kinases 1 and 2 (MSK1/2) (Deak *et al.* 1998).

Stress-activated protein kinases (JNK and p38)

The kinases JNK and p38 MAPK are collectively known as stress-activated protein kinases because of their activation by environmental stressors, including osmotic and heat shock (Galcheva-Gargova *et al.* 1994, Kyriakis *et al.* 1994, Cuenda *et al.* 1997), stretch (Komuro *et al.* 1996), or inflammatory cytokines (Han *et al.* 1994, Kyriakis *et al.* 1994). The upstream kinases, MEK3, MEK4 (SEK1), MEK6 and MEK7 (Jiang *et al.* 1997, Keesler *et al.* 1998, Hu *et al.* 1999) phosphorylate p38 MAPK on threonine and tyrosine residues at the sequence Thr–Gly–Tyr, resulting in p38 MAPK activation. The SEK1/MEK4 is a potent activator of SAPK1/JNK and SAPK2/p38 MAPK *in vitro* and *in vivo*, and is highly expressed in skeletal muscle (Sanchez *et al.* 1994). Further upstream, the Rho family of low molecular weight GTP-binding proteins, Rac and Cdc42, are identified as potential regulators of the p38 MAPK pathway (Zhang *et al.* 1995). Phosphorylated p38 MAPK activates downstream targets including MAPKAPK 2 (Rouse *et al.* 1994), and MSK1/2 (Deak *et al.* 1998).

Transcription factors activated by MAPK pathways

In addition to cytoplasmic proteins, which after activation undergo translocation to the nucleus, nuclear proteins can also be phosphorylated by MAPK (Chen *et al.* 1992). Putative nuclear targets include transcription factors, indicating the importance of MAPK in the regulation of transcriptional activity (Karin 1995). Transcription factors activated by ERK1/2, JNK and/or p38 MAPK include cyclic AMP response element binding protein (CREB), Elk-1, activating transcription factor (ATF) 1 and 2, myocyte enhance factor 2 (MEF2) A and C, c-fos and c-jun (Kyriakis *et al.* 1994, Cavigelli *et al.* 1995, Gupta *et al.* 1995, Zhang *et al.* 1995, Xing *et al.* 1996, Han *et al.* 1997). These transcription factors are involved in the control and induction of gene expression and are usually induced very early in a variety of intra- and extracellular signals.

The CREB binds to the cyclic AMP response element (CRE) and activates transcription in response to a variety of extracellular signals including cAMP, increased intracellular Ca^{2+}, as well as several growth factors (Gonzalez & Montminy 1989, Sheng *et al.* 1991, Ginty *et al.* 1994, Kwok *et al.* 1994). In neural cells, ERK signal transduction is necessary for Ca^{2+}-stimulated transcription, via a pathway that involves phosphorylation and activation of CREB (Impey *et al.* 1998). As muscle contraction is dependent upon Ca^{2+}-release from the sarcoplasmic reticulum (SR), CREB may be an intracellular target that undergoes phosphorylation in response to exercise.

Exercise-induced adaptations in skeletal muscle

Signal transduction in contracting muscle may be altered by; (1) increased blood flow (Armstrong & Laughlin 1984) leading to increased delivery of hormonal factors to the muscle, which may activate receptor-mediated signalling (2) release of autocrine and paracrine factors from the contracting muscle (Stebbins *et al.* 1990, Perrone *et al.* 1995, O'Neill *et al.* 1996), which might stimulate cell surface receptors and thereby activate the MAPK cascade (3) contraction *per se*. During and after physical activity and exercise, the internal milieu of the skeletal muscle will undergo changes, which will disturb the cellular homeostasis (Booth & Thomason 1991). Such molecular and cellular responses to exercise may transduce signals for adaptations because of exercise training. Several factors, many of which are interdependent, have been proposed. These factors include: factors causing fatigue, such as ATP depletion (Shoubridge *et al.* 1985), reduced cross-bridge formation and Ca^{2+} cycling, decreased pH associated with lactate accumulation (Hermansen &

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Osnes 1972), glycogen depletion (Bergstrom *et al.* 1967), and possibly impaired maximal oxygen flux (Fig. 2). Certain intramuscular consequences of exercise have been brought forward as possible regulators of training-induced adaptations: changes in intracellular $[Ca^{2+}]$ (Byrd *et al.* 1989, Sreter *et al.* 1987), elevated cAMP levels (Kraus *et al.* 1989), hypoxia (Terrados *et al.* 1990), mechanical stretch (Holly *et al.* 1980), fibre recruitment pattern (Henriksson & Reitman 1976), and possibly reactive oxygen species (ROS) and general substrate flux.

Muscle damage/stress may also elicit signal transduction. Exercise-induced muscle damage, as determined by myofibril disruption (Friden *et al.* 1981) or elevated serum concentrations of creatine kinase (Clarkson *et al.* 1986), is generally greater in eccentric vs. concentric exercise. Muscle damage in response to eccentric vs. concentric exercise may be attributed to different stress stimuli. Potentially, concentric-induced muscle damage is primarily because of metabolic stress, whereas eccentric-induced muscle damage is caused by fibre disruption that leads to metabolic stress factors. However, exercise responses are unlikely because of one single mechanism, more likely they depend on the integration of multiple systemic and/or local factors. Nerves, cytokines, autocrine and paracrine substances, hormones, temperature, circulatory changes and fluid shifts within the muscle that alter the concentration of factors may be involved in the adaptation to exercise (Fig. 2).

MAPK signal transduction in response to exercise

Mitrogen-activated protein kinase signalling pathways have been implicated in the regulation of gene transcription in numerous cell types (Kyriakis *et al.* 1994, Cavigelli *et al.* 1995, Gupta *et al.* 1995, Xing *et al.* 1996). Importantly, recent evidence suggests that activation of MAPK signal-transduction cascade(s) regulate gene expression in response to exercise or muscle contraction elicited by electrical stimulation in rat (Table 2) and human (Table 3) skeletal muscle.

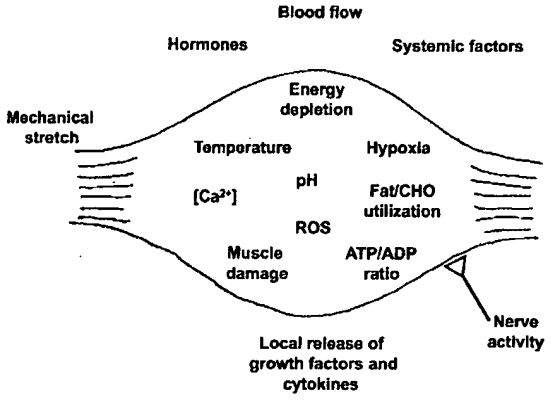

Figure 2 Proposed factors involved in exercise-induced adaptations in skeletal muscle. These factors are of extra-, as well as intracellular origin. Exercise responses may be because of a combination of several of these factors, and do not depend on one single mechanism. CHO – carbohydrates, ROS – reactive oxygen species.

ERK1/2 MAPK

In human skeletal muscle, exercise (acute cycling) leads to a profound increase in ERK1/2 phosphorylation (Aronson *et al.* 1997b, Widegren *et al.* 1998), with the greatest effect noted after 30 min of continuous cycling at 75% $VO_{2\,max}$ (Widegren *et al.* 1998). Exercise-induced MAPK phosphorylation is rapidly decreased upon cessation of exercise, and is completely restored to resting levels 60 min after exercise (Widegren *et al.* 1998). This exercise-induced ERK1/2 phosphorylation cannot be attributed to an increase in total ERK1/2 protein expression (Widegren *et al.* 1998). The MAPK signalling pathways are activated by different stress stimuli (Galcheva-Gargova *et al.* 1994, Kyriakis *et al.* 1994), therefore the observed exercise-induced ERK1/2 phosphorylation may represent a biopsy-sampling artifact. However, repeated biopsy sampling from different incisions does not alter ERK1/2 phosphorylation, providing evidence that increased ERK1/2 phosphorylation is a genuine exercise effect (Widegren *et al.* 1998). Nevertheless, ERK1/2 phosphorylation is increased in human skeletal muscle when two biopsies are obtained from the same incision (Aronson *et al.* 1998b). Thus, important care needs to be given to the biopsy sampling protocol when studying MAPK activation in human muscle.

The ERK1/2 phosphorylation is only observed in exercised muscle, providing support that local rather than systemic factors are mediating this effect (Aronson *et al.* 1997b, Widegren *et al.* 1998). Yet in one study, a measurable increase in ERK1/2 phosphorylation was not noted in exercising muscle until after 60 min of cycle ergometry (90% of the subjects anaerobic threshold, or workload corresponding to ~60% of $VO_{2\,max}$) (Osman *et al.* 2000). The lack of a measurable effect of exercise on ERK1/2 phosphorylation after 30 min in the latter study may depend on different antibodies and assays used for analysis, different exercise protocols and/or differences in age and physical fitness ($VO_{2\,max}$) of the study participants. Exercise-effects on MAPK signalling are intensity dependent, as ERK1/2 phosphorylation is greater in high vs. low intensity exercise (Widegren *et al.* 2000). Overall, these findings support the hypothesis that ERK1/2 is involved in exercise-induced adaptations as low-intensity short-term (30 min) exercise can adequately

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Table 2 Summary of published reports regarding exercise/contraction effects on mitogen-activated protein kinase (MAPK) signal transduction in rat skeletal muscle

Species	Exercise model	Upstream kinases	MAPK response	Downstream kinases	Reference
Rat	Treadmill running for 5 weeks		ERK1 mRNA ↑ ERK2 mRNA ⇔ ERK3 mRNA ⇔		Kim *et al.* (1995)
Rat	10–60 min of treadmill running		ERK1/2 ↑ ^{p}p38 MAPK ↑ 145% of basal ^{a}JNK 2-fold ↑	^{a}RSK2 1.8-fold ↑	Goodyear *et al.* (1996)
Rat	Sciatic nerve stimulation	^{a}MEKK1 2-fold ↑ at 60 min ^{p}MEK4 3.5-fold ↑at 30 min ^{a}MEK1 4-fold ↑ at 15 s	^{a}JNK 5-fold ↑ at 30 min ^{p}JNK 2-fold ↑ at 30 min ^{p}ERK1/2 14-fold ↑at 10 min	c-jun mRNA 2-fold ↑ c-fos mRNA 20-fold ↑	Aronson *et al.* (1997a)
Rat	Sciatic nerve stimulation	^{e}Raf-1 ↑ ^{a}MEK1 ↑	^{p}ERK1/2 6-fold ↑	^{a}RSK2 4-fold ↑ c-fos mRNA 25-fold ↑ ^{e}p70^{S6K} ⇔	Sherwood *et al.* (1999)
Rat	Perfused hindlimb, sciatic nerve stimulation		^{p}ERK1 wg 5-fold, rg 2.5-fold, s2-fold ↑ ^{p}ERK2 wg 13-fold, rg 4-fold, s4-fold ↑		Wojtaszewski *et al.* (1999)
Rat	Isolated contractions		^{p}ERK1 2.9-fold ↑ ^{p}ERK2 2.4-fold ↑ ^{p}ERK1/2 2–3-fold ↑	^{a}p90^{rsk} 5-fold ↑	Ryder *et al.* (2000)
Rat	Isolated contractions		^{p}p38 MAPK ↑	^{a}MAPKAPK2 10-fold ↑ ^{a}MSK1 5-fold ↑	
Rat	*In vivo* transfection of re-generating muscle, electrical stimulation	Ras mutants	^{a}ERK 6-fold ↑	Slow MHC expression ↑	Murgia *et al.* (2000)
Rat	Isolated contractions		^{p}ERK1/2 1.5–1.8-fold ↑ ^{p}p38 MAPK 1.5–3-fold ↑		Wretman *et al.* (2000)
Rat	Isometric contractions (IC), static stretch (SS)		IC: ^{a}JNK 1.8-fold ↑, ^{p}p38 MAPK 4-fold ↑, ^{p}ERK1/2 1.6-fold ↑ SS: ^{a}JNK 19-fold ↑, ^{p}p38 MAPK 21-fold ↑, ^{p}ERK1/2 2-fold ↑		Boppart *et al.* (2000)

p – phosphorylation, a – activity, e – electrophoretic mobility, wg – white gastrocnemius, rg – red gastrocnemius, s – soleus.

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Table 3 Summary of published reports of exercise effects on mitogen-activated protein kinase (MAPK) signal transduction in human skeletal muscle

Species	Exercise model	Upstream kinases	MAPK response	Downstream kinases	Reference
Human	60 min of cycling at 70% of $VO_{2\ max}$	^{a}Raf-1 2-fold ↑ ^{a}MEK1 3-fold ↑	^{p}ERK1/2 4–24-fold ↑	^{a}RSK 1.6–4.6-fold ↑	Aronson *et al.* (1997a)
Human	One-leg cycling at 70% of $VO_{2\ max}$ for 60 min	^{p}SEK1 2.5-fold ↑	^{p}ERK1/2 31-fold ↑ ^{p}p38 MAPK 2-fold ↑	^{p}CREB ↑ in non-working muscle	Widegren *et al.* (1998)
Human	60 min of cycling at 70% of $VO_{2\ max}$		^{a}JNK 6-fold ↑	c-jun mRNA 1.8-fold ↑	Aronson *et al.* (1998)
Human	Concentric (C) and eccentric (E) exercise	^{a}MKK4 C and E: 2-fold ↑	^{a}JNK C = 4-fold, E = 15-fold ↑		Boppart *et al.* (1999)
Human	Cycling at 60% of $VO_{2\ max}$ for 30 min, n = 2 for 60 min	^{a}MEK1 ⇔	^{p}ERK1/2 ⇔ for 30 min, 2-fold ↑ for 60 min ^{a}ERK2 ⇔ for 30 min, 2-fold ↑ for 60 min	^{a}RSK2 ⇔	Osman *et al.* (2000)
Human	Marathon running	^{p}MKK4 4-fold ↑ ^{p}MKK6 1.5-fold ↑	^{a}JNK 7-fold ↑ ^{a}p38 MAPK α ⇔, γ 1.5-fold ↑ ^{p}p38 MAPK α ⇔, γ 4-fold ↑		Boppart *et al.* (2000)
Human	One-leg cycling at 40% and 75% of $VO_{2\ max}$ for 30 min	^{p}MEK1/2 2.5-, and 4.8-fold ↑	^{p}ERK1/2 11.5-, and 39-fold ↑	^{p}CREB ⇔ ^{a}MSK1 4–5-fold ↑	Widegren *et al.* (2000)
Human	One-leg cycling at 70% of $VO_{2\ max}$ for 60 min			^{a}MSK2 2–3-fold ↑ ^{a}p90^{rsk} 5-fold ^{a}MAPKAPK2 3.3-fold	Krook *et al.* (2000)
Human	Trained vs. untrained		Total ERK1/2 expression 1.9-fold ↑ Total p38 MAPK expression 32% ↓		Yu *et al.* (2001)

p – phosphorylation, a – activity, e – electrophoretic mobility.

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increase ERK1/2 signalling (Aronson *et al.* 1997b, Widegren *et al.* 1998, 2000).

The ERK1/2 phosphorylation in response to exercise/contraction appears to involve the Raf-1, MEK1 signalling pathway in human (Aronson *et al.* 1997b, Widegren *et al.* 2000) and rat skeletal muscle (Sherwood *et al.* 1999). However, the upstream events in the exercise-induced signalling cascade remain elusive. In rat skeletal muscle, conventional and novel PKCs do not appear to be involved in contraction-induced ERK1/2 phosphorylation (Hayashi *et al.* 1999, Ryder *et al.* 2000). Furthermore, contraction-induced MAPK signalling does not involve activation of receptor tyrosine kinases (Sherwood *et al.* 1999). Likewise, contraction-induced activation of ERK1/2 appears to be independent of IRS and Shc tyrosine phosphorylation and of formation of Shc/Grb2 or IRS1/Grb2 complexes (Sherwood *et al.* 1999) providing evidence that activation of MAPK signalling by exercise/muscle contraction is not mediated via classical receptor tyrosine kinase pathways. Additionally, G_i-protein complexes are not involved as a link in the contraction-mediated ERK phosphorylation (J.R. Zierath and J.W. Ryder, unpublished observation).

Effects of exercise on stress-activated protein kinases

The effects of physical exercise in human subjects is not limited to the activation of the ERK signalling cascade, as activation of p38 MAPK and JNK has also been observed in human skeletal muscle (Aronson *et al.* 1997b, Widegren *et al.* 1998). The p38 MAPK, together with JNK, are classified as stress-activated protein kinases. Recent evidence suggests that JNK and p38 MAPK, and their upstream regulators, MEK4 and MEK6, are transiently increased after prolonged strenuous physical activity (marathon running) (Boppart *et al.* 2000). These findings are important because they suggest stress-activated protein kinase cascades may be responsible for skeletal muscle adaptation to strenuous exercise.

p38 MAPK

The p38 MAPK signal transduction can be mediated by four isoforms (α, β, δ, γ) (Keesler *et al.* 1998, Hu *et al.* 1999). The existence of these different isoforms is likely to give specificity in mediating down-stream biological responses induced by exercise. For example, p38γ is highly expressed in human skeletal muscle and although it does not activate ATF-2 or MAPKAP-K2, it does phosphorylate myelin basic protein (Li *et al.* 1996). After marathon running, p38γ phosphorylation has been reported to be elevated four-fold, whereas p38α phosphorylation and activity are not altered (Boppart *et al.* 2000).

The time-course for exercise-induced p38 phosphorylation has been determined in human skeletal muscle following one-leg cycle ergometry at ~70% $VO_{2\,max}$ (Widegren *et al.* 1998). Exercise increased p38 MAPK phosphorylation after 30 min, with phosphorylation sustained at 60 min The magnitude of p38 phosphorylation was less than observed for ERK1/2. In contrast to ERK1/2, p38 MAPK phosphorylation was increased in non-exercised muscle upon cessation of exercise, suggesting p38 MAPK is mediated by both local and systemic factors associated with exercise. However, the nature of this circulating factor has yet to be identified.

JNK

Exercise also elicits an increase in JNK activity in human skeletal muscle (Aronson *et al.* 1998a, Boppart *et al.* 1999), with the greatest effect observed in response to eccentric exercise (Boppart *et al.* 1999). Injury-producing exercise induces several stimuli, including inflammatory cytokines (Fielding *et al.* 1993), which may act as a trigger to elicit responses via the stress-activated protein kinase cascades. Clearly, MAPK activation by exercise is likely to be dependent on the type of exercise and the extent of muscle injury. Interestingly, both JNK and ERK1/2 activity are increased in response to sciatic nerve stimulation of rat hind-limb muscles (Aronson *et al.* 1997a). However, the contraction response for these two kinases revealed differential time courses. This might indicate activation by different exercise-induced stimuli.

Activation of downstream MAPK targets in response to exercise

Several downstream substrates of MAPK have been identified (Brunet & Pouyssegur 1997), including MSK1 and 2 (Deak *et al.* 1998), p90rsk, and MAPKAP-K2 (Zhao *et al.* 1996, Stugill *et al.* 1988). Recent studies have provided evidence to delineate the effects of exercise on these MAPK targets in skeletal muscle (Ryder *et al.* 2000, Krook *et al.* 2000b).

MSK1 and MSK2

In cultured cells MSK1 and 2 are directly activated by ERK1/2 and p38 MAPK, as determined by *in vitro* and *in vivo* assays (Deak *et al.* 1998). Both MSK1 and MSK2 are downstream targets of the MAPK cascades that are activated in response to muscle contraction and exercise (Ryder *et al.* 2000, Krook *et al.* 2000b). Specific inhibitors of ERK and p38 MAPK signalling pathways (PD98059 and SB203580, respectively) have been powerful tools to determine the downstream components of the MAPK signalling cascades in response to exercise/contraction (Ryder *et al.* 2000). In isolated muscle, PD98059 and

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SB203580, independently and completely inhibited contraction-stimulated MSK1 activity (Ryder *et al.* 2000). Thus, both ERK1/2 and p38 MAPK are required for contraction-mediated MSK1 activation. In human skeletal muscle, exercise elicits a rapid increase in MSK1 and MSK2 activity with peak activation observed after 60 min (Krook *et al.* 2000b). This activation is specific to exercised muscle, consistent with previous reports for ERK1/2 (Widegren *et al.* 1998). Thus, MSK1 and MSK2 are activated by local rather than systemic factors in response to exercise. As the pattern of exercise-induced activation of MSK1 and MSK2 (Krook *et al.* 2000a) closely follows the activation pattern of ERK1/2, rather than p38 MAPK (Widegren *et al.* 1998), exercise-mediated activation of MSK1 and MSK2 in human muscle is likely to occur primarily via an ERK1/2-dependent pathway. Activated MSK1 has been shown to phosphorylate histone H3/HMG-14 (Thomson *et al.* 1999), as well as the transcription factor CREB (Deak *et al.* 1998), suggesting a role of this kinase in the regulation of gene expression.

p90rsk and MAPKAPK2

The effects of muscle contraction/exercise on p90rsk (Zhao *et al.* 1996) and MAPKAPK2 (Cuenda *et al.* 1995, Beyaert *et al.* 1996) have also been determined (Goodyear *et al.* 1996, Aronson *et al.* 1997b, Ryder *et al.* 2000, Krook *et al.* 2000b). These enzymes are downstream targets of ERK and p38 MAPK, respectively, and may act as mediators between signal transduction pathways and intranuclear events. In isolated rat epitrochlearis muscle, *in vitro* contraction-induced activation of p90rsk parallels ERK1/2 phosphorylation (Ryder *et al.* 2000). Inhibition of ERK by PD98059 completely abolished the effect of contraction on p90rsk activity (Ryder *et al.* 2000), suggesting muscle contraction induced p90rsk activity is ERK-dependent. Consistent with this observation, the exercise-induced activation of p90rsk (Krook *et al.* 2000b) follows the phosphorylation pattern of ERK1/2 in human muscle (Widegren *et al.* 1998). This finding is consistent with p90rsk as a downstream target of ERK1/2 (Ryder *et al.* 2000).

The MAPKAPK2 is a downstream target of p38 MAPK (Cuenda *et al.* 1995, Hazzalin *et al.* 1996, Ryder *et al.* 2000). In rat skeletal muscle, inhibition the p38 MAPK pathway by SB203580 ablates contraction-induced MAPKAPK2 activity (Ryder *et al.* 2000). Clearly inhibitor studies are not possible to perform in exercising humans. However, the finding that exercise increases MAPKAPK2 activity in human skeletal muscle (Krook *et al.* 2000b) is consistent with a previous report using electrical stimulation of rat muscle (Ryder *et al.* 2000). The finding of exercise-mediated activation of p90rsk and MAPKAPK2 activity in skeletal muscle offers support for the hypothesis that contraction-induced MAPK signalling increases transcriptional activity, as these kinases have been implicated in the phosphorylation of transcription factors (Cohen 1997).

Exercise-induced signal transduction: implications for transcriptional responses

Exercise has numerous growth and metabolic effects in skeletal muscle, including changes in glycogen metabolism, glucose and amino acid uptake, protein synthesis and gene transcription. However, the mechanisms by which exercise regulates intracellular signal transduction to the transcriptional machinery in the nucleus, thus modulating gene expression, are largely unknown. Chronic increases in physical activity can lead to muscle hypertrophy and increased expression of numerous muscle proteins (Goldberg *et al.* 1975, Booth & Thomason 1991, Ren *et al.* 1994, Chibalin *et al.* 2000, Yu 2001). These activity-induced changes in muscle phenotype may be regulated partly by the induction of immediate-early genes including c-fos and c-jun (Michel *et al.* 1994). Activation of JNK and ERK1/2 by contraction/exercise is also associated with rapid induction of c-jun and c-fos, as evidenced by increased mRNA levels (Aronson *et al.* 1997a, Aronson *et al.* 1998a, Boppart *et al.* 1999). This supports the notion of the MAPK cascade as a possible upstream regulator of early response genes by contraction/exercise. Thus, exercise-inducing signals communicate with this regulatory machinery through intracellular cascades, which leads to the activation of different transcription factors responsible for starting gene expression and protein synthesis (Fig. 3).

CONCLUSION

Mitogen-activated protein kinase cascades have been implicated as possible signalling mechanisms involved in the regulation of exercise-induced adaptations. A variety of acute and chronic changes in the muscle phenotype occur in response to moderate exercise. Importantly, exercise is a powerful activator of MAPK cascades and several downstream enzymes. The profound exercise-induced increase in phosphorylation of ERK1/2 is rapid and declines to basal levels after cessation of exercise. The exercise effect on ERK1/2 is limited to working muscle, providing evidence for local rather than systemic factors mediating phosphorylation. Furthermore, exercise-mediates p38 MAPK phosphorylation. This response might depend on systemic factors, as an increased phosphorylation can also be observed in non-exercising muscle. Exercise appears to favour ERK1/2 over p38 MAPK signalling, as exercise

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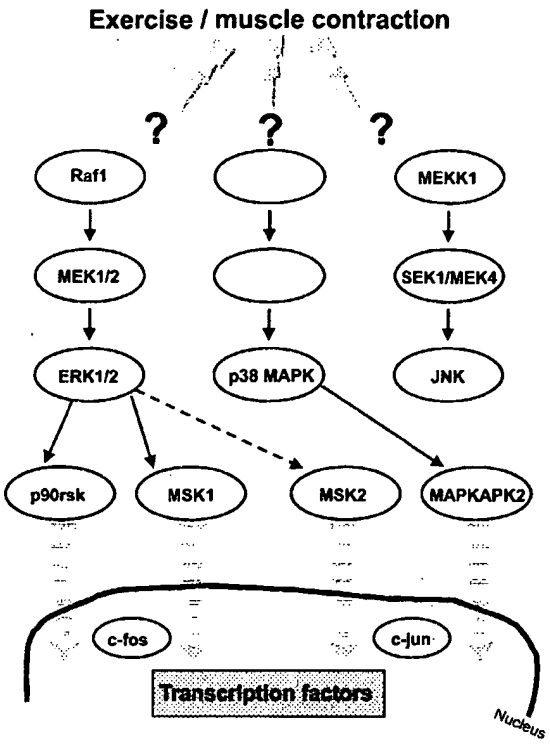

Figure 3 Mitogen-activated protein kinase (MAPK) targets are activated in skeletal muscle in response to acute exercise or contractions.

effects on p38 MAPK are less robust. However, the direct link between exercise and MAPK signalling is not fully defined. Specific inhibitors of ERK and p38 MAPK may be useful in the future to determine whether MAPK signalling cascades are directly involved in transcriptional regulation of important genes in skeletal muscle in response to exercise.

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REFERENCES

Aikawa, R., Komuro, I., Yamazaki, T. *et al.* 1997. Oxidative stress activates extracellular signal-regulated kinases through Src and Ras in cultured cardiac myocytes of neonatal rats. *J Clin Invest* **100,** 1813–1821.

Anderson, N.G., Maller, J.L., Tonks, N.K. & Sturgill, T.W. 1990. Requirement for integration of signals from two distinct phosphorylation pathways for activation of MAP kinase. *Nature* **343,** 651–653.

Armstrong, R.B. & Laughlin, M.H. 1984. Exercise blood flow patterns within and among rat muscles after training. *Am J Physiol* **246,** H59–H68.

Aronson, D., Dufresne, S.D. & Goodyear, L.J. 1997a. Contractile activity stimulates the c-Jun NH2-terminal kinase pathway in rat skeletal muscle. *J Biol Chem* **272,** 25 636–25 640.

Aronson, D., Violan, M.A., Dufresne, S.D., Zangen, D., Fielding, R.A. & Goodyear, L.J. 1997b. Exercise stimulates the mitogen-activated protein kinase pathway in human skeletal muscle. *J Clin Invest* **99,** 1251–1257.

Aronson, D., Boppart, M.D., Dufresne, S.D., Fielding, R.A. & Goodyear, L.J. 1998a. Exercise stimulates c-Jun NH2 kinase activity and c-Jun transcriptional activity in human skeletal muscle. *Biochem Biophys Res Commun* **251,** 106–110.

Aronson, D., Wojtaszewski, J.F., Thorell, A., Nygren, J., Zangen, D., Richter, E.A., Ljungqvist, O., Fielding, R.A. & Goodyear, L.J. 1998b. Extracellular-regulated protein kinase cascades are activated in response to injury in human skeletal muscle. *Am J Physiol* **275,** C555–C561.

Bergstrom, J., Hermansen, L., Hultman, E. & Saltin, B. 1967. Diet, muscle glycogen and physical performance. *Acta Physiol Scand* **71,** 140–150.

Beyaert, R., Cuenda, A., Vanden Berghe, W., Plaisance, S., Lee, J.C., Haegeman, G., Cohen, P. & Fiers, W. 1996. The p38/RK mitogen-activated protein kinase pathway regulates interleukin-6 synthesis response to tumor necrosis factor. *Embo J* **15,** 1914–1923.

Booth, F.W. & Thomason, D.B. 1991. Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. *Physiol Rev* **71,** 541–585.

Boppart, M.D., Aronson, D., Gibson, L., Roubenoff, R., Abad, L.W., Bean, J., Goodyear, L.J. & Fielding, R.A. 1999. Eccentric exercise markedly increases c-Jun NH (2) - terminal kinase activity in human skeletal muscle. *J Appl Physiol* **87,** 1668–1673.

Boppart, M.D., Asp, S., Wojtaszewski, J.F., Fielding, R.A., Mohr, T. & Goodyear, L.J. 2000. Marathon running transiently increases c-Jun NH2-terminal kinase and p38 activities in human skeletal muscle. *J Physiol (Lond)* **526,** 663–669.

Boulton, T.G., Nye, S.H., Robbins, D.J., Ip, N.Y., Radziejewska, E., Morgenbesser, S.D., DePinho, R.A., Panayotatos, N., Cobb, M.H. & Yancopoulos, G.D. 1991. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* **65,** 663–675.

Brunet, A. & Pouyssegur, J. 1997. Mammalian MAP kinase modules: how to transduce specific signals. *Essays Biochem* **32,** 1–16.

Byrd, S.K., Bode, A.K. & Klug, G.A. 1989. Effects of exercise of varying duration on sarcoplasmic reticulum function. *J Appl Physiol* **66,** 1383–1389.

Cavigelli, M., Dolfi, F., Claret, F.X. & Karin, M. 1995. Induction of c-fos expression through JNK-mediated TCF/Elk-1 phosphorylation. *EMBO J* **14,** 5957–5964.

Chen, R.H., Sarnecki, C. & Blenis, J. 1992. Nuclear localization and regulation of ERK- and Rsk-encoded protein kinases. *Mol Cell Biol* **12,** 915–927.

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Chibalin, A.V., Yu, M., Ryder, J.W., Song, X.M., Galuska, D., Krook, A., Wallberg-Henriksson, H. & Zierath, J.R. 2000. Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: differential effects on insulin-receptor substrates 1 and 2. *Proc Natl Acad Sci USA* **97**, 38–43.

Clarkson, P.M., Byrnes, W.C., McCormick, K.M., Turcotte, L.P. & White, J.S. 1986. Muscle soreness and serum creatine kinase activity following isometric, eccentric, and concentric exercise. *Int J Sports Med* **7**, 152–155.

Cohen, P. 1997. The search for physiological substrates of MAP and SAP kinases in mammalian cells. *Trends Cell Biol* **7**, 353–360.

Cooper, J.A. & Hunter, T. 1983. Identification and characterization of cellular targets for tyrosine protein kinases. *J Biol Chem* **258**, 1108–1115.

Crespo, P., Xu, N., Simonds, W.F. & Gutkind, J.S. 1994. Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits. *Nature* **369**, 418–420.

Cuenda, A., Cohen, P., Buee-Scherrer, V. & Goedert, M. 1997. Activation of stress-activated protein kinase-3 (SAPK3) by cytokines and cellular stresses is mediated via SAPKK3 (MKK6); comparison of the specificities of SAPK3 and SAPK2 (RK/p38). *EMBO J* **16**, 295–305.

Cuenda, A., Rouse, J., Doza, Y.N., Meier, R., Cohen, P., Gallagher, T.F., Young, P.R. & Lee, J.C. 1995. SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett* **364**, 229–233.

Deak, M., Clifton, A.D., Lucocq, L.M. & Alessi, D.R. 1998. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *EMBO J* **17**, 4426–4441.

Downward, J. 1996. Control of ras activation. *Cancer Surv* **27**, 87–100.

Fielding, R.A., Manfredi, T.J., Ding, W., Fiatarone, M.A., Evans, W.J. & Cannon, J.G. 1993. Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. *Am J Physiol* **265**, R166–R172.

Force, T. & Bonventre, J.V. 1998. Growth factors and mitogen-activated protein kinases. *Hypertension* **31**, 152–161.

Friden, J., Sjostrom, M. & Ekblom, B. 1981. A morphological study of delayed muscle soreness. *Experientia* **37**, 506–507.

Galcheva-Gargova, Z., Derijard, B., Wu, I.H. & Davis, R.J. 1994. An osmosensing signal transduction pathway in mammalian cells. *Science* **265**, 806–808.

Ginty, D.D., Bonni, A. & Greenberg, M.E. 1994. Nerve growth factor activates a Ras-dependent protein kinase that stimulates c-fos transcription via phosphorylation of CREB. *Cell* **77**, 713–725.

Goldberg, A.L., Etlinger, J.D., Goldspink, D.F. & Jablecki, C. 1975. Mechanism of work-induced hypertrophy of skeletal muscle. *Med Sci Sports* **7**, 185–198.

Gonzalez, G.A. & Montminy, M.R. 1989. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* **59**, 675–680.

Goodyear, L.J., Chang, P.Y., Sherwood, D.J., Dufresne, S.D. & Moller, D.E. 1996. Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. *Am J Physiol* **271**, E403–E408.

Gupta, S., Campbell, D., Derijard, B. & Davis, R.J. 1995. Transcription factor ATF2 regulation by the JNK signal transduction pathway. *Science* **267**, 389–393.

Han, J., Jiang, Y., Li, Z., Kravchenko, V.V. & Ulevitch, R.J. 1997. Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. *Nature* **386**, 296–299.

Han, J., Lee, J.D., Bibbs, L. & Ulevitch, R.J. 1994. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* **265**, 808–811.

Hayashi, T., Hirshman, M.F., Dufresne, S.D. &.Goodyear, L.J. 1999. Skeletal muscle contractile activity in vitro stimulates mitogen-activated protein kinase signaling. *Am J Physiol* **277**, C701–C707.

Hazzalin, C.A., Cano, E., Cuenda, A., Barratt, M.J., Cohen, P. & Mahadevan, L.C. 1996. p38/RK is essential for stress-induced nuclear responses: JNK/SAPKs and c-Jun/ATF-2 phosphorylation are insufficient. *Curr Biol* **6**, 1028–1031.

Henriksson, J. & Reitman, J.S. 1976. Quantitative measures of enzyme activities in type I and type II muscle fibres of man after training. *Acta Physiol Scand* **97**, 392–397.

Hermansen, L. & Osnes, J.B. 1972. Blood and muscle pH after maximal exercise in man. *J Appl Physiol* **32**, 304–308.

Hodge, C., Liao, J., Stofega, M., Guan, K., Carter-Su, C. & Schwartz, J. 1998. Growth hormone stimulates phosphorylation and activation of elk-1 and expression of c-fos, egr-1, and junB through activation of extracellular signal-regulated kinases 1 and 2. *J Biol Chem* **273**, 31 327–31 336.

Holly, R.G., Barnett, J.G., Ashmore, C.R., Taylor, R.G. & Mole, P.A. 1980. Stretch-induced growth in chicken wing muscles: a new model of stretch hypertrophy. *Am J Physiol* **238**, C62–C71.

Houmard, J.A., Egan, P.C., Neufer, P.D., Friedman, J.E., Wheeler, W.S., Israel, R.G. & Dohm, G.L. 1991. Elevated skeletal muscle glucose transporter levels in exercise-trained middle-aged men. *Am J Physiol* **261**, E437–E443.

Houmard, J.A., Shaw, C.D., Hickey, M.S. & Tanner, C.J. 1999. Effect of short-term exercise training on insulin-stimulated PI 3- kinase activity in human skeletal muscle. *Am J Physiol* **277**, E1055–E10560.

Hu, M.C., Wang, Y.P., Mikhail, A., Qiu, W.R. & Tan, T.H. 1999. Murine p38-delta mitogen-activated protein kinase, a developmentally regulated protein kinase that is activated by stress and proinflammatory cytokines. *J Biol Chem* **274**, 7095–7102.

Hughes, V.A., Fiatarone, M.A., Fielding, R.A., Kahn, B.B., Ferrara, C.M., Shepherd, P., Fisher, E.C., Wolfe, R.R., Elahi, D. & Evans, W.J. 1993. Exercise increases muscle GLUT-4 levels and insulin action in subjects with impaired glucose tolerance. *Am J Physiol* **264**, E855-E862.

Ichijo, H., Nishida, E., Irie, K., ten Dijke, P., Saitoh, M., Moriguchi, T., Takagi, M., Matsumoto, K., Miyazono, K. & Gotoh, Y. 1997. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* **275**, 90–94.

Impey, S., Obrietan, K., Wong, S.T., Poser, S., Yano, S., Wayman, G., Deloulme, J.C., Chan, G. & Storm, D.R.

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1998. Cross talk between ERK and PKA is required for Ca^{2+} stimulation of CREB- dependent transcription and ERK nuclear translocation. *Neuron* **21,** 869–883.

Jiang, Y., Gram, H., Zhao, M., New, L., Gu, J., Feng, L., Di Padova, F., Ulevitch, R.J. & Han, J. 1997. Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38delta. *J Biol Chem* **272,** 30 122–30 128.

Karin, M. 1995. The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* **270,** 16 483–16 486.

Keesler, G.A., Bray, J., Hunt, J., Johnson, D.A., Gleason, T., Yao, Z., Wang, S.W., Parker, C., Yamane, H., Cole, C. & Lichenstein, H.S. 1998. Purification and activation of recombinant p38 isoforms alpha, beta, gamma, and delta. *Protein Expr Purif* **14,** 221–228.

Kim, Y., Inoue, T., Nakajima, R. *et al.* 1995. Effects of endurance training on gene expression of insulin signal transduction pathway. *Biochem Biophys Res Commun* **210,** 766–773.

Komuro, I., Kudo, S., Yamazaki, T., Zou, Y., Shiojima, I. & Yazaki, Y. 1996. Mechanical stretch activates the stress-activated protein kinases in cardiac myocytes. *FASEB J* **10,** 631–636.

Kraus, W.E., Bernard, T.S. & Williams, R.S. 1989. Interactions between sustained contractile activity and beta-adrenergic receptors in regulation of gene expression in skeletal muscles. *Am J Physiol* **256,** C506–C514.

Krook, A., Björnholm, M., Galuska, D., Jiang, X.-J., Fahlman, R., Myers, M.G. Jr., Wallberg-Henriksson, H. & Zierath, J.R. 2000a. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabetes* **49,** 284–292.

Krook, A., Widegren, U., Jiang, X.J., Henriksson, J., Wallberg-Henriksson, H., Alessi, D. & Zierath, J.R. 2000b. Effects of exercise on mitogen- and stress-activated kinase signal transduction in human skeletal muscle. *Am J Physiol* **279,** R1716–R1721.

Kwok, R.P., Lundblad, J.R., Chrivia, J.C., Richards, J.P., Bachinger, H.P., Brennan, R.G., Roberts, S.G., Green, M.R. & Goodman, R.H. 1994. Nuclear protein CBP is a coactivator for the transcription factor CREB. *Nature* **370,** 223–226.

Kyriakis, J.M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E.A., Ahmad, M.F., Avruch, J. & Woodgett, J.R. 1994. The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* **369,** 156–160.

Leppa, S., Saffrich, R., Ansorge, W. & Bohmann, D. 1998. Differential regulation of c-Jun by ERK and JNK during PC12 cell differentiation. *EMBO J* **17,** 4404–4013.

Li, Z., Jiang, Y., Ulevitch, R.J. & Han, J. 1996. The primary structure of p38γ: a new member of p38 group of MAP kinase. *Biochem Biophys Res Commun* **228,** 334–340.

Michel, J.B., Ordway, G.A., Richardson, J.A. & Williams, R.S. 1994. Biphasic induction of immediate early gene expression accompanies activity-dependent angiogenesis and myofiber remodeling of rabbit skeletal muscle. *J Clin Invest* **94,** 277–285.

Murgia, M., Serrano, A.L., Calabria, E., Pallafacchina, G., Lomo, T. & Schiaffino, S. 2000. Ras is involved in nerve-activity-dependent regulation of muscle genes. *Nat Cell Biol* **2,** 142–147.

Ogata, A., Chauhan, D., Teoh, G., Treon, S.P., Urashima, M., Schlossman, R.L. & Anderson, K.C. 1997. IL-6 triggers cell growth via the Ras-dependent mitogen-activated protein kinase cascade. *J Immunol* **159,** 2212–2221.

O'Neill, C.A., Stebbins, C.L., Bonigut, S., Halliwell, B. & Longhurst, J.C. 1996. Production of hydroxyl radicals in contracting skeletal muscle of cats. *J Appl Physiol* **81,** 1197–1206.

Osman, A.A., Pendergrass, M., Koval, J., Maezono, K., Cusi, K., Pratipanawatr, T. & Mandarino, L.J. 2000. Regulation of MAP kinase pathway activity in vivo in human skeletal muscle. *Am J Physiol Endocrinol Metab* **278,** E992–E999.

Pages, G., Lenormand, P., L'Allemain, G., Chambard, J.C., Meloche, S. & Pouyssegur, J. 1993. Mitogen-activated protein kinases p42MAPK and p44MAPK are required for fibroblast proliferation. *Proc Natl Acad Sci USA* **90,** 8319–8323.

Perrone, C.E., Fenwick-Smith, D. & Vandenburgh, H.H. 1995. Collagen and stretch modulate autocrine secretion of insulin-like growth factor-1 and insulin-like growth factor binding proteins from differentiated skeletal muscle cells. *J Biol Chem* **270,** 2099–2106.

Ploug, T., van Deurs, B., Ai, H., Cushman, S.W. & Ralston, E. 1998. Analysis of GLUT4 distribution in whole skeletal muscle fibers: Identification of distinct storage compartments that are recruited by insulin and muscle contractions. *J Cell Biol* **142,** 1429–1446.

Ray, L.B. & Sturgill, T.W. 1988. Insulin-stimulated microtubule-associated protein kinase is phosphorylated on tyrosine and threonine in vivo. *Proc Natl Acad Sci USA* **85,** 3753–3777.

Ren, J.M., Semenkovich, C.F., Gulve, E.A., Gao, J. & Holloszy, J.O. 1994. Exercise induces rapid increases in GLUT4 expression, glucose transport capacity, and insulin-stimulated glycogen storage in muscle. *J Biol Chem* **269,** 14 396–14 401.

Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamazares, A., Zamanillo, D., Hunt, T. & Nebreda, A.R. 1994. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell* **78,** 1027–1037.

Ryder, J.W., Fahlman, R., Wallberg-Henriksson, H., Alessi, D.R., Krook, A. & Zierath, J.R. 2000. Effect of contraction on mitogen-activated protein kinase signal transduction in skeletal muscle. Involvement of the mitogen- and stress-activated protein kinase 1. *J Biol Chem* **275,** 1457–1462.

Sanchez, I., Hughes, R.T., Mayer, B.J., Yee, K., Woodgett, J.R., Avruch, J., Kyriakis, J.M. & Zon, L.I. 1994. Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun. *Nature* **372,** 794–798.

Schlaepfer, D.D., Hanks, S.K., Hunter, T. & van der Geer, P. 1994. Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature* **372,** 786–791.

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Schonwasser, D.C., Marais, R.M., Marshall, C.J. & Parker, P.J. 1998. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. *Mol Cell Biol* **18,** 790–798.

Seger, R., Ahn, N.G., Posada, J., Munar, E.S., Jensen, A.M., Cooper, J.A., Cobb, M.H. & Krebs, E.G. 1992. Purification and characterization of mitogen-activated protein kinase activator (s) from epidermal growth factor-stimulated A431 cells. *J Biol Chem* **267,** 14 373–14 381.

Seko, Y., Tobe, K., Ueki, K., Kadowaki, T. & Yazaki, Y. 1996. Hypoxia and hypoxia/reoxygenation activate Raf-1, mitogen-activated protein kinase kinase, mitogen-activated protein kinases, and S6 kinase in cultured rat cardiac myocytes. *Circ Res* **78,** 82–90.

Sheng, M., Thompson, M.A. & Greenberg, M.E. 1991. CREB: a Ca(2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases. *Science* **252,** 1427–1430.

Sherwood, D.J., Dufresne, S.D., Markuns, J.F., Cheatham, B., Moller, D.E., Aronson, D. & Goodyear, L.J. 1999. Differential regulation of MAP kinase, p70 (S6K), and Akt by contraction and insulin in rat skeletal muscle. *Am J Physiol* **276,** E870–E878.

Shoubridge, E.A., Challiss, R.A., Hayes, D.J. & Radda, G.K. 1985. Biochemical adaptation in the skeletal muscle of rats depleted of creatine with the substrate analogue beta-guanidinopropionic acid. *Biochem J* **232,** 125–131.

Skolnik, E.Y., Batzer, A., Li, N., Lee, C.H., Lowenstein, E., Mohammadi, M., Margolis, B. & Schlessinger, J. 1993. The function of GRB2 in linking the insulin receptor to Ras signaling pathways. *Science* **260,** 1953–1955.

Sreter, F.A., Lopez, J.R., Alamo, L., Mabuchi, K. & Gergely, J. 1987. Changes in intracellular ionized Ca concentration associated with muscle fiber type transformation. *Am J Physiol* **253,** C296–C300.

Stancato, L.F., Sakatsume, M., David, M., Dent, P., Dong, F., Petricoin, E.F., Krolewski, J.J., Silvennoinen, O., Saharinen, P., Pierce, J., Marshall, C.J., Sturgill, T., Finbloom, D.S. & Larner, A.C. 1997. Beta interferon and oncostatin M activate Raf-1 and mitogen-activated protein kinase through a JAK1-dependent pathway. *Mol Cell Biol* **17,** 3833–3840.

Stebbins, C.L., Carretero, O.A., Mindroiu, T. & Longhurst, J.C. 1990. Bradykinin release from contracting skeletal muscle of the cat. *J Appl Physiol* **69,** 1225–1230.

Stugill, T.W., Ray, L.B., Erikson, E. & Maller, J.L. 1988. Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. *Nature* **334,** 715–718.

Terrados, N., Jansson, E., Sylven, C. & Kaijser, L. 1990. Is hypoxia a stimulus for synthesis of oxidative enzymes and myoglobin? *J Appl Physiol* **68,** 2369–2372.

Thomson, S., Clayton, A.L., Hazzalin, C.A., Rose, S., Barratt, M.J. & Mahadevan, L.C. 1999. The nucleosomal response associated with immediate-early gene induction is mediated via alternative MAP kinase cascades: MSK1 as a potential histone H3/HMG-14 kinase. *EMBO J* **18,** 4779–4793.

Wang, P.H., Schaaf, G.J., Chen, W.H., Feng, J., Prins, B.A., Levin, E.R. & Bahl, J.J. 1998. IGF I induction of p53 requires activation of MAP kinase in cardiac muscle cells. *Biochem Biophys Res Commun* **245,** 912–917.

Widegren, U., Jiang, X.J., Krook, A., Chibalin, A.V., Bjornholm, M., Tally, M., Roth, R.A., Henriksson, J., Wallberg-Henriksson, H. & Zierath, J.R. 1998. Divergent effects of exercise on metabolic and mitogenic signaling pathways in human skeletal muscle. *FASEB J* **12,** 1379–1389.

Widegren, U., Wretman, C., Lionikas, A., Hedin, G. & Henriksson, J. 2000. Influence of exercise intensity on ERK/MAP kinase signalling in human skeletal muscle. *Pflugers Arch Eur J Physiol* **441,** 317–322.

Widmann, C., Gibson, S., Jarpe, M.B. & Johnson, G.L. 1999. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev* **79,** 143–180.

Winston, L.A. & Hunter, T. 1996. Intracellular signalling: putting JAKs on the kinase MAP. *Curr Biol* **6,** 668–671.

Wojtaszewski, J.F., Lynge, J., Jakobsen, A.B., Goodyear, L.J. & Richter, E.A. 1999. Differential regulation of MAP kinase by contraction and insullin in skeletal muscle: metabolic implications. *Am J Physiol* **277,** E724–E732.

Wretman, C., Widegren, U., Lionikas, A., Westerblad, H. & Henriksson, J. 2000. Differential activation of mitogen-activated protein kinase signalling pathways by isometric contractions in isolated slow- and fast-twitch rat skeletal muscle. *Acta Physiol Scand* **170,** 45–49.

Xing, J., Ginty, D.D. & Greenberg, M.E. 1996. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. *Science* **273,** 959–963.

Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Mizuno, T., Takano, H., Hiroi, Y., Ueki, K., Tobe, K. *et al.* 1995. Mechanical stress activates protein kinase cascade of phosphorylation in neonatal rat cardiac myocytes. *J Clin Invest* **96,** 438–446.

Yamazaki, T., Tobe, K., Hoh, E., Maemura, K., Kaida, T., Komuro, I., Tamemoto, H., Kadowaki, T., Nagai, R. & Yazaki, Y. 1993. Mechanical loading activates mitogen-activated protein kinase and S6 peptide kinase in cultured rat cardiac myocytes. *J Biol Chem* **268,** 12069–12076.

Yu, M., Blomstrand, E., Chibalin, A.V., Wallberg-Henriksson, H., Zierath, J.R. & Krook, A. 2001. Exercise-associated difference in an array of proteins involved in signal transduction and glucose transport. *J Appl Physiol* **90,** 29–34.

Zhang, S., Han, J., Sells, M.A., Chernoff, J., Knaus, U.G., Ulevitch, R.J. & Bokoch, G.M. 1995. Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1. *J Biol Chem* **270,** 23934–23936.

Zhao, Y., Bjorback, C. & Moller, D.E. 1996. Regulation and interaction of pp90 (rsk) isoforms with mitogen-activated protein kinases. *J Biol Chem* **271,** 29773–29779.

Zheng, C.F. & Guan, K.L. 1993. Properties of MEKs, the kinases that phosphorylate and activate the extracellular signal-regulated kinases. *J Biol Chem* **268,** 23933–23939.

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different effectors amongst which Raf, PI3K and RalGDS are the most characterized. However, the consequences of a quantitative modulation of Ras activity on downstream signaling pathways and how this can lead to various biological responses are still not fully understood. Thus, in order to investigate the molecular dynamics of Ras signaling network, we first studied Raf pathway induction. In vivo monitoring of Ras-Raf interaction by protein-fragment complementation assay as well as in vitro detection of activated Ras and ERK show that Raf pathway activation follows different kinetics according to the level of stimulus at the cell surface. We are now exploring other interactions of Ras signaling network in order to have a better understanding of how the regulation of signal transduction at a quantitative level can influence signaling specificity and lead to various responses at a qualitative level.

377.4

SMAD/Sp network regulates IGFBP-5 transcription

Vincenzo Cesi, Fabiola Sesti, Barbara Tanno, Roberta Vitali, Maria Laura Giuffrida, Camillo Mancini, Giuseppe Raschella. Toxicology and Biomedical Sciences, Ente Nuove tecnologie Energia Ambiente (ENEA), via Anguillarese, 301, Rome, 00060 Italy

The Insulin-like Growth Factor Binding Protein 5 (IGFBP-5) belongs to the family of the IGF Binding Proteins, which is involved in the modulation of IGFs binding ability to their receptor (IGF1R). Deletion analysis allowed us to highlight a proximal region of the promoter which is essential to IGFBP-5 transcription. Here we describe the players involved in this regulation by functional and binding assays. The TGF-β superfamily is formed by TGF-β and BMPs which exert different cell-type specific functions that are mediated by specific SMAD family members. The proximal region of IGFBP-5 promoter (-330 to –1) contains multiple putative core binding sites for transcription factors. Our experiments demonstrate the existence of two closely spaced binding sites for SMAD and Sp factors within this region. Using EMSA and DAPA techniques we isolated a 42 bp transcriptional unit (-173 to –132) which is a binding site for Sp factors and both TGF-β- and BMP-regulated SMADs. We characterized the Sp and SMAD family members that bind this transcriptional unit and other partners of the transcription complex. By differentiation experiments, we determined which factors are involved in the differentiation-driven IGFBP-5 upregulation. Functional assays demonstrated that recombinant proteins of the TGF-β superfamily and inhibitors of PI3K/AKT and $p70^{S6K}$ modulate IGFBP-5 transcription.

377.5

Gene Therapy: Medicine of the 21st Century

Inder Verma. Laboratory of Genetics, The Salk Institute, 10010 North Torrey Pines Rd., La Jolla, CA 92037

Presently gene therapy is being contemplated for both genetic and acquired diseases. A variety of somatic tissues are being explored for the introduction of foreign genes with a view towards gene therapy. A prime requirement for successful gene therapy is the sustained expression of the therapeutic gene without any adverse effect on the recipient. A highly desirable delivery vehicle will be one that can be generated at high amounts, integrate in non-dividing cells and have little or no associated immune problems. We have generated vectors based on the AIDS virus that have the ability to introduce genes into both dividing and non-dividing cells. The vectors (lentiviral) also have an expanded host range and can introduce genes in a variety of cells. We have generated third generation of lentiviral packaging constructs that contain only the gag/pol, VSV G envelope and the sin vector. Thus our current lentiviral vectors are devoid of six viral genes, therefore we consider them to be safe vectors. Using third generation lentiviral vectors we can introduce genes directly into brain, liver, muscle, hematopoietic stem cells, retina, and a number of tumor cells. Our data shows that lentiviral vectors can not only efficiently deliver genes, but also have long term sustained production of the foreign protein. We have not observed any untoward immunological consequences due to the vector.

377.6

Plasma membrane phospholipid scramblase 1 promotes EGF-dependent activation of c-Src and potentiates signaling through EGFR and related growth factor receptors

Meera Nanjundan, Jun Sun, Peter J. Sims, Therese Wiedmer. Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Phospholipid scramblase 1 (PLSCR1) is a palmitoylated, endofacial membrane protein proposed to mediate transbilayer movement of plasma membrane PLs. PLSCR1 is a component of membrane lipid rafts and has been shown to both physically and functionally interact with EGFR. Cell stimulation by EGF results in Tyr phosphorylation of PLSCR1, its association with Shc and EGFR, and rapid cycling of PLSCR1 between plasma membrane and endosomal compartments. Consistent with its potential role in growth factor signaling pathways, granulocyte precursors from mice deficient in PLSCR1 show impaired proliferation and maturation under cytokine stimulation. Using PLSCR1-/- embryonic fibroblasts, we demonstrate that deletion of PLSCR1 from the plasma membrane reduces activation of c-Src by EGF, suggesting that PLSCR1 normally facilitates receptor-dependent activation of c-Src. We also show that upon EGF stimulation, PLSCR1 is phosphorylated by c-Src, within the tandem repeat sequence ^{68}VYNQPVYNQP^{77}. In addition to attenuating c-Src activation, deletion of PLSCR1 was accompanied by diminished phosphorylation of signaling kinases including c-Src, Erk, and Akt. Attenuated phosphorylation in PLSCR1-/- cells was also observed in response to PDGF, suggesting that PLSCR1 serves as a common adapter that promotes downstream signaling through multiple growth factor receptors. Supported by NIH grant HL36946, HL63819, and HL61200.

377.7

Investigation of BMK1/ERK5 Mitogen Activated Protein (MAP) kinase signaling using a conditional knockout mouse model

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Our lab has identified BMK1/ERK5, a recently discovered mammalian MAP kinase, as a central mitogenic-signaling molecule. The physiological function of BMK1 pathway has been implicated in cell proliferation, cell differentiation and cell survival. On the other hand, the pathological role of this pathway has been implicated in carcignogenesis, cardiac hypertrophy and reperfusion ischemia. So far, knowledge of the mechanism of action and the role of BMK1 pathway has been mostly derived from in vitro cell-based experiments. Lately, two other labs have produced a traditional BMK1 knockout mouse model in which the BMK1 gene is deleted prior to conception resulting in embryonic lethality. This early death of the BMK1-null mutant precluded the functional study of BMK1 pathway in adult animals. In order to study BMK1's function in mature mice, we report here the generation of a conditional knockout mouse model for the BMK1 gene in which the expression of the BMK1 gene is under the control of the Cre-Lox system. The mice with homozygous BMK1-floxed alleles developed normally with similar expression level of BMK1 protein compared with their wild-type littermates. By mating with transgenic mice carrying Cre recombinase, the deletion of the BMK1 gene in BMK1-floxed animals has been achieved in specific cell types and at specific time points. This experimental approach should allow us to define the function of BMK1 signaling pathway in animal development, physiology or behavior.

377.8

Profiling of Transcriptional Targets Through a Combinatorial Approach

Wendy J. Freebern[1], Cynthia M. Haggerty[1], Markey C. McNutt[1], James L. Smith[1], Paul J. Hoover[1], Idalia Montano[1], Kevin Gardner[2]. [1]Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, NIH, 8717 Grovemont Circle, Bethesda, MD 20892-4605, [2]Laboratory of Pathology and Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, NIH, Bethesda, MD

Tightly controlled cellular processes from proliferation to apoptosis occur through complicated networks of transcriptional regulation. The

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Mitogen-Activated Protein Kinase: Conservation of a Three-Kinase Module From Yeast to Human

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I. INTRODUCTION: DISCOVERY THAT MITOGEN-ACTIVATED PROTEIN KINASE^{ERK} IS REGULATED BY PHOSPHORYLATION ON THREONINE AND TYROSINE

In the early 1980s, it was realized in several different cell types stimulated with growth factors including platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) that a predominant protein phosphorylated on tyrosine had a size of 42 kDa (59, 61). Proteins of the same size and behavior on two-dimensional gels were phosphorylated on tyrosine in response to phorbol esters (174), in virus-transformed cells (60), and in metaphase-arrested *Xenopus laevis* eggs (57). At the same time, ser-

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ine-threonine protein kinases activated by the insulin receptor tyrosine kinase were being characterized (7, 287, 294). The hypothesis was considered that direct regulation by tyrosine phosphorylation catalyzed by the insulin receptor would regulate the activity of serine-threonine protein kinases (7, 287). Ray and Sturgill (287) demonstrated that a 42-kDa serine-threonine protein kinase, referred to as mitogen-activated protein kinase (MAPK), isolated from insulin-stimulated 3T3-L1 cells was phosphorylated on both threonine and tyrosine. It was quickly realized that the 42-kDa MAPK characterized to be threonine and tyrosine phosphorylated in response to insulin was the same protein shown to be tyrosine phosphorylated in response to other growth factors, phorbol esters, viral transformation, and metaphase arrest in *Xenopus* eggs (294). It was also demonstrated that phosphorylation of both the threonine and tyrosine was required for MAPK activation (294).

The cDNA encoding MAPK was isolated by Boulton et al. (32) who renamed it ERK1 for extracellular signal-regulated kinase 1, because of the variety of extracellular signals that could stimulate its activity. The isolation of cDNA for ERK2 and ERK3 quickly followed (31). Alignment of the ERK sequences showed they were closely related to the *Saccharomyces cerevisiae* protein kinases Fus3 and Kss1, demonstrating the close homology between mammalian and yeast MAPK.

Biochemical characterization and molecular cloning identified the upstream kinases in the $MAPK^{erk}$ module in mammalian cells (67). The work defined a conserved three-kinase module in mammals. The MAPK regulatory system is a three-kinase module that establishes a sequential protein kinase activation pathway.

In parallel, geneticists were identifying the component genes in yeast mating. The sterile (ste) genes in *S. cerevisiae* involved in pheromone-induced mating were ordered and cloned (136). Quickly, the related genes in *Schizosaccharomyces pombe* were identified and their protein products characterized (260). Cumulatively, this work identified a conserved three-component protein kinase module that included the MAPK Fus3 and Kss1 in *S. cerevisiae* and Spk1 in *S. pombe*.

In this review, we detail the properties of the known MAPK modules. The expanding number of MAPK modules and their role in controlling complex cellular functions defines their importance in responsiveness of cells and organisms to their environment.

II. MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS: ACTIVATION MODULES INVOLVING THREE KINASES

Pathways involving MAPK are activated in response to an extraordinarily diverse array of stimuli. These stimuli vary from growth factors and cytokines to irradiation, osmolarity, and the shear stress of fluid flowing over a cell. The basic assembly of MAPK pathways is a three-component module conserved from yeast to humans. The minimal MAPK module is composed of three kinases that establish a sequential activation pathway (Fig. 1). The first kinase of the three-component activation module is a MAPK kinase kinase (MKKK) (92). Specific MKKK have been shown to be activated either by phosphorylation by a MAPK kinase kinase kinase (MKKKK) or by interaction with a small GTP-binding protein of the Ras or Rho family. Other potential modes of activation include oligomerization and subcellular relocalization. The MKKK are serine/threonine kinases that when activated phosphorylate and activate the next kinase in the module, a MAPK kinase (MKK) (315). The MKK are kinases that recognize and phosphorylate a Thr-X-Tyr motif in the activation loop of MAPK (109), defining MKK as dual-specificity kinases. Mitogen-activated protein kinases are the final kinase in the three-kinase module and phosphorylate substrates on serine and threonine residues. The vast majority of defined substrates for MAPK are transcription factors. However, MAPK have the ability to phosphorylate many other substrates including other protein kinases, phospholipases, and cytoskeleton-associated proteins.

Why have MAPK activation modules evolved having three kinases? The reason probably lies in the unique activation properties of MAPK. Mitogen-activated protein kinase must be phosphorylated on both a threonine and

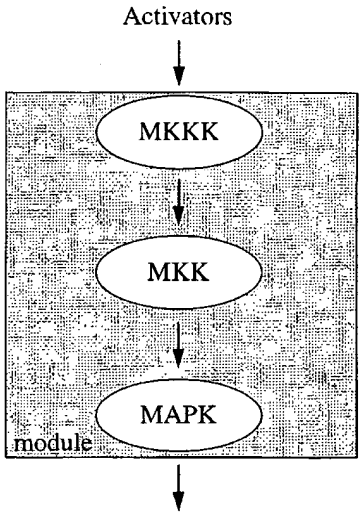


FIG. 1. Core module of a mitogen-activated protein kinase (MAPK) pathway is composed of 3 kinases [MAPK kinase kinase (MKKK), MAPK kinase (MKK), and MAPK] that are sequentially activated by phosphorylation.

tyrosine for their activation, a dual phosphorylation catalyzed by a specific MKK. The different MKK recognize a tertiary structure of specific MAPK and not simply a linear sequence surrounding the Thr-X-Tyr activation motif of MAPK. As described in this review, very specific MKK and MAPK combinations are found in a MAPK module. Specific MKK appear to recognize the tertiary structure of different MAPK, effectively restricting their regulation of different MAPK subtypes. In contrast, MKKK are able to mix and match with different MKK-MAPK combinations. In mammalian cells, there are more known MKKK than MAPK. The MKK represent the fewest members of the three-component MAPK modules. The completion of the human genome project will be required to define the exact number of kinases in each of these groups. The large number of MKKK allows for diversity of inputs from numerous stimuli to feed into specific MAPK pathways. Some kinases that appear to be MKKK may regulate pathways not involving MAPK [such as regulation of the NFκB pathway by MEKK1 (139, 196, 233, 393)]. Thus the regulation of MAPK pathways at the level of MKKK may represent branch points in regulation of signal pathways in some cases.

Table 1 lists the members of the known MAPK three-component modules defined to date. Perusal of Table 1 boggles the mind and clearly demonstrates that the current nomenclature, or more appropriately the lack of nomenclature, in the MAPK field poses a major problem of understanding the MAPK literature, especially for readers not familiar with the field. In the present review, we decided to name a given component of a MAPK module according to its position in the pathway with the current name of the protein in superscript. For example, the yeast Ste11 MAPK kinase kinase will be described as $MKKK^{ste11}$. This notation should greatly facilitate the reading of this review, since it is not required to know the identity of each kinase to determine its position in a given MAPK pathway.

To date, 14 MKKK, 7 MKK, and 12 MAPK have been identified in mammalian cells (Table 1). Dendogram analysis indicates that these kinases belong to different subfamilies (Fig. 2). Four subfamilies among the MKKK can currently be defined. The Raf subfamily is the best characterized and comprises $MKKK^{B\text{-}raf}$, $MKKK^{A\text{-}raf}$, and $MKKK^{raf1}$. The MEK kinase (MEKK) subfamilly is made of the four MEKK ($MKKK^{mekk1-4}$). $MKKK^{ask1}$ and $MKKK^{tpl2}$ appear to form a third MKKK subfamily. The fourth group in the dendogram is more diverse and comprises $MKKK^{mst}$, $MKKK^{sprk}$, $MKKK^{muk}$, $MKKK^{tak1}$, and the most distantly related MKKK, $MKKK^{mos}$. For the MKK, MKK^{mek1} and MKK^{mek2} are closely related as are MKK^{mkk3} and MKK^{mkk6}. The MAPK can be categorized into five subfamilies: the $MAPK^{erk1/2}$, the $MAPK^{p38}$, the $MAPK^{jnk}$, the $MAPK^{erk5}$, and the $MAPK^{erk3/4}$ subfamilies. These MAPK give the name to the MAPK pathways that employ them (i.e., the MAPK pathways using the $MAPK^{jnk}$ are called the JNK MAPK pathways). This review focuses on the organization, regulation, and function of the different MAPK modules in eukaryotic cells.

III. ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASES: DUAL PHOSPHORYLATION OF THE ACTIVATION LOOP

Mitogen-activated protein kinases are proline directed in that they only phosphorylate substrates that contain a proline in the P-1 site (219). A general consensus for $MAPK^{erk1/2}$ is Pro-X-Ser/Thr-Pro (6). The activity of MAPK is controlled by dual phosphorylation in an amino acid sequence known as the activation loop. The sequence Thr-X-Tyr in the activation loop, where X can be different amino acids among the MAPK, is the site for dual phosphorylation catalyzed by specific MKK (4). For $MAPK^{erk1/2}$, the phosphorylation sites correspond to Thr-183 and Tyr-185. Dual phosphorylation of these sites results in a >1,000-fold increase in specific activity of the MAPK.

The core three-dimensional structure of protein kinases, as resolved from the crystal structure of protein kinase A (PKA), is composed of two domains with the active site at the domain interface (177). Adenosine 5′-triphosphate binds in the active site cleft, and peptide substrate for some kinases has been shown to bind in a groove on the surface of the COOH-terminal domain of the active site (178). The phosphoreceptor amino acid (Ser, Thr, Tyr) of the substrate binds near the catalytic loop in this domain. A surface loop contiguous with this COOH-terminal domain is found in many protein kinases and encodes a phosphorylation site; this sequence is referred to as the activation loop or lip. Threonine-183 in $MAPK^{erk1/2}$ is homologous to the phosphorylation site in the activation loop of other protein kinases (165). The tyrosine phosphorylation site in the activation loop of MAPK is unique.

The recent crystal structure of active, dual-phosphorylated $MAPK^{erk2}$ has been recently solved (40). Phosphorylation of Thr-183 and Tyr-185 in the activation loop causes the loop to refold and interact with surface arginine-binding sites. The conformational changes in the activation loop and neighboring sequences result in activation of the kinase.

An interesting property of the requirement for dual phosphorylation of the Thr-X-Tyr sequence is that substitution of the Thr and Tyr by acidic amino acids like glutamate do not result in constitutive activation. The characteristics of the activation loop and the dual phosphorylation of the Thr and Tyr induce several conformational changes in the protein that cannot be mimicked by glutamates. For this reason, point mutations in MAPK do not activate their activity. The requirement for the P-Thr-X-P-

TABLE 1. *Components of MAPK pathways*

Species	Type	Current Names	Nomenclature in This Review	Homologs/Isoforms	Reference No.
		Lower eukaryotes			
Saccharomyces cerevisiae	MKKK	STE11	$MKKK^{ste11}$		45
		BCK1	$MKKK^{bck1}$		200
		SSK2	$MKKK^{ssk2}$		220
		SSK22	$MKKK^{ssk22}$		220
	MKK	STE7	MKK^{ste7}		45
		MKK1	MKK^{mkk1}		154
		MKK2	MKK^{mkk2}		154
		PBS2	MKK^{pbs2}		24
	MAPK	FUS3	$MAPK^{fus3}$		84
		KSS1	$MAPK^{kss1}$		63
		MPK1, SLT2, SLK2, BYC2	$MAPK^{mpk1}$		339
		SMK1	$MAPK^{smk1}$		185
		HOG1	$MAPK^{hog1}$		35
		YKL161C[c]	$MAPK^{ykl161c}$		147
Schyzosaccharomyces pombe	MKKK	Byr2	$MKKK^{byr2}$		359
		Wik1, Wak1, Wis4	$MKKK^{wik1}$		313
		Win1	$MKKK^{win1}$		299
	MKK	Byr1	MKK^{byr1}		257
		Wis1	MKK^{wis1}		360
	MAPK	Spk1	$MAPK^{spk1}$		338
		Spm1	$MAPK^{spm1}$		395
		Sty1, Spc1, Phh1	$MAPK^{sty1}$		171
		Pmk1, Spm1	$MAPK^{pmk1}$		337
Candida albicans	MKK	CaSte7	$caMKK^{ste7}$		314
	MAPK	MKC1	$caMAPK^{mkc1}$		258
		ERK1	$caMAPK^{crk1}$		364
Fusarium solani	MAPK	FsMAPK	$fsMAPK^{mapk}$		207
		Higher eukaryotes (mammals, birds, amphibians, and fishes)			
	MKKK	c-Raf, Raf-1, c-Raf-1 (187)	$MKKK^{raf1}$	$hMKKK^{raf1}$	26
				$rMKKK^{raf1}$	155
				$xMKKK^{raf1}$	202
		Mos (276)	$MKKK^{mos}$	$hMKKK^{mos}$	363
				$rMKKK^{mos}$	346
				$ggMKKK^{mos}$	304
				$xMKKK^{mos}$	102, 297
		MEKK1 (191)	$MKKK^{mekk1}$	$mMKKK^{mekk1}$	191
				$rMKKK^{mekk1}$	385
		B-Raf, c-Rmil (343)	$MKKK^{B\text{-}raf}$	$hMKKK^{B\text{-}raf}$	316
				$mMKKK^{B\text{-}raf}$	235
				$ggMKKK^{B\text{-}raf}$	39
				$ccoMKKK^{B\text{-}raf}\ \alpha$[d]	91
				$ccoMKKK^{B\text{-}raf}\ \beta$[d]	91
		TAK1 (387)	$MKKK^{tak1}$	$mMKKK^{tak1}$	387
		A-Raf (379)	$MKKK^{A\text{-}raf}$	$hMKKK^{A\text{-}raf}$	16, 198
				$rMKKK^{A\text{-}raf}$	156
		Tpl-2, Cot, Est (44,298)	$MKKK^{tpl2}$	$hMKKK^{tpl2}\alpha^1$	8, 9, 243
				$hMKKK^{tpl2}\beta^1$	9
				$mMKKK^{tpl2}$	268
				$rMKKK^{tpl2}$	273
		MEKK2 (20)	$MKKK^{mekk2}$	$mMKKK^{mekk2}$	20
		MEKK3 (20)	$MKKK^{mekk3}$	$hMKKK^{mekk3}$	86
				$mMKKK^{mekk3}$	20
		MUK, DLK, ZPK (137)	$MKKK^{muk}$	$hMKKK^{muk}$	288, 289
				$mMKKK^{muk}$	142
				$rMKKK^{muk}$	137
		SPRK, PTK-1, MLK-3 (286)	$MKKK^{sprk}$	$hMKKK^{sprk}$	106, 150
		MAPKKK5, ASK1 (149,357)	$MKKK^{ask1}$	$hMKKK^{ask1}$	357
				$mMKKK^{ask1}$	336
		MEKK4, MTK1 (111)	$MKKK^{mekk4}$	$hMKKK^{mekk4}$	330
				$mMKKK^{mekk4}\alpha$	111
				$mMKKK^{mekk4}\beta$	111
		MST, MLK2 (138)	$MKKK^{mst}$	$hMKKK^{mst}$	79, 173
	MKK	MKK1, MPK1, MEK, MEK1, ERK activator kinase 1	MKK^{mek1}	$hMKK^{mek1}\alpha$[e]	402
				$hMKK^{mek1}\beta$[e]	309
				$mMKK^{mek1}$	66
				$rMKK^{mek1}$	78, 269, 378

TABLE 1—*Continued*

Species	Type	Current Names	Nomenclature in This Review	Homologs/Isoforms	Reference No.
Higher eukaryotes (mammals, birds, amphibians, and fishes) (Continued)					
				cgMKK^{mek1}	271
				ocMKK^{mek1}	10
				xMKK^{mek1}	182
	MKK	MKK2, MPK2, MEK2, ERK activator kinase 2	MKK^{mek2}	hMKK^{mek2}	402
				mMKK^{mek2}	38
				rMKK^{mek2}	269, 377
				ccaMKK^{mek2}	146
		MKK3, MPK3, SAPKK2	MKK^{mkk3}	hMKK^{mkk3}	74
				mMKK^{mkk3}	248
		MKK4, SEK1, SEK, MPK4, SAPKK1, JNKK	MKK^{mkk4}	hMKK^{mkk4}	74
				mMKK^{mkk4}	300
				xMKK$^{mkk4}\alpha^{b}$	392
				xMKK$^{mkk4}\beta^{b}$	392
		MEK5, MPK5	MKK^{mek5}	hMKK^{mek5}	405
				rMKK$^{mek5}\alpha$	88
				rMKK$^{mek5}\beta$	88
		MKK6, MEK6, MPK6, SAPKK3, SSK3	MKK^{mkk6}	hMKK^{mkk6}	322
				mMKK^{mkk6}	68
		MKK7, JNKK2	MKK^{mkk7}	hMKK^{mkk7}	382
				mMKK$^{mkk7}\alpha$	141
				mMKK$^{mkk7}\beta$	141
	MAPK	ERK1, p44 MAPK, MBP kinase	MAPK^{erk1}	hMAPK^{erk1}	49, 270
				mMAPK^{erk1}	65, 70
				rMPAK^{erk1}	32
		ERK2, MAPK2, ERT1, p42 MAPK	MAPK^{erk2}	hMAPK^{erk2}	118, 270
				mMAPK^{erk2}	90
				rMAPK^{erk2}	31, 134
				xMAPK^{erk2}	120
		ERK3, p55 MAPK (rat), p97 MAPK (human)	MAPK^{erk3}	hMAPK^{erk3}	231, 406
				rMAPK^{erk3}	31
		ERK4, MNK2, p63 MAPK	MAPK^{erk4}	hMAPK^{erk4}	118
				rMAPK^{erk4}	107
		ERK5, BMK	MAPK^{erk5}	h MAPK^{erk5}	405
		JNK1, SAPK-1, p54γ SAPK	MAPK^{jnk1}	hMAPK$^{jnk1}\alpha1$	123
				hMAPK$^{jnk1}\alpha2$	123
				hMAPK$^{jnk1}\beta1$	123
				hMAPK$^{jnk1}\beta2$	123
				rMAPK$^{jnk1}\alpha2$	188
				ccaMAPK$^{jnk1}\alpha2$	132
				ccaMAPK$^{jnk1}\beta2$	132
		JNK2, p54α SAPK	MAPK^{jnk2}	hMAPK$^{jnk2}\alpha1$	123
				hMAPK$^{jnk2}\alpha2$	123, 169
				hMAPK$^{jnk2}\beta1$	123
				hMAPK$^{jnk2}\beta2$	123
				rMAPK$^{jnk2}\alpha2$	188
				rMAPK$^{jnk2}\beta2$	188
				ggMAPK$^{jnk2}\alpha1$	157
		JNK3, p54β SAPK	MAPK^{jnk3}	hMAPK$^{jnk3}\alpha1$	123
				hMAPK$^{jnk3}\alpha2$	123
				mMAPK$^{jnk3}\alpha1$	228
				mMAPK$^{jnk3}\alpha2$	228
				rMAPK$^{jnk3}\alpha2$	188
		p38α, p38 MAPK, p38/HOG1, MPK2, SAPK-2, RK, RK/p38, p40, CSBP1, CSBP2, Mxi2, CRK1	MAPK$^{p38\alpha}$	hMAPK$^{p38\alpha}\alpha^{g}$	105, 197
				hMAPK$^{p38\alpha}\beta^{g}$	197
				hMAPK$^{p38\alpha}\gamma^{g}$	397
				mMAPK$^{p38\alpha}$	128
				xMAPK$^{p38\alpha}$	295
		p38β, p38-2	MAPK$^{p38\beta}$	hMAPK$^{p38\beta}$	162, 323
		p38γ, SAPK-3, ERK6	MAPK$^{p38\gamma}$	hMAPK$^{p38\gamma}$	116, 195
				rMAPK$^{p38\gamma}$	232
		p38δ, SAPK-4	MAPK$^{p38\delta}$	hMAPK$^{p38\delta}$	115, 358
				mMAPK$^{p38\delta}$	163

(*Continued*)

TABLE 1—*Continued*

Species	Type	Current Names	Nomenclature in This Review	Homologs/Isoforms	Reference No.
		Higher eukaryotes (others)			
Arabidopsis thaliana	MKKK	ATMEKK1	atMKKK^{mekk1}		246
		ANP1	atMKKK$^{anp1}\alpha$[h]		264
			atMKKK$^{anp1}\beta$[h]		264
		ANP2	atMKKK^{anp2}		264
		ANP3	atMKKK^{anp3}		264
	MAPK	ATMPK1	atMAPK^{mpk1}		244
		ATMPK2	atMAPK^{mpk2}		244
		ATMPK3	atMAPK^{mpk3}		245
		ATMPK4	atMAPK^{mpk4}		245
		ATMPK5	atMAPK^{mpk5}		245
		ATMPK6	atMAPK^{mpk6}		245
		ATMPK7	atMAPK^{mpk7}		245
Avena sativa (oat)	MAPK	Aspk9, AsMAP1	asMAPK^{aspk9}		148
Caenorhabditis elegans	MKKK	Lin-45	cMKKK^{lin45} (328)		129
	Ksr-1	cMKKK^{ksr1}			
		CELR03G5[f]	cMKKK^{r03g5}		373
	MKK	MEK-2	cMKK^{mek2}		181, 381
	MAPK	Sur-1, Mpk-1	cMAPK^{sur1}		189, 380
		CELC04G6[f]	cMAPK^{c04g6}		373
		CELB0478[f]	cMAPK^{b0478}		373
		CELB0218[f]	cMAPK^{b0218}		373
Dictyostelium discoideum	MKK	DdMEK1	ddMKK^{mek1}		216
	MAPK	DdERK1	ddMAPK^{erk1}		110
Drosophila melanogaster	MKKK	Draf-1	dMKK^{raf1}		263
	MKK	Hep	dMKK^{hep}		114
		Dsor1	dMKK^{dsor1}		341
		D-MKK3	dMKK^{mkk3}		130
		D-MKK4	dMKK^{mkk4}		130
	MAPK	DJNK	dMAPK^{bsk}		292
		DmERK-A	dMAPK^{rolled}		19
		Dp38a	dMAPK^{p38a}		126
		Dp38b	dMAPK^{p38b}		130
Entamoeba histolytica	MKKK	ehMos	ehMKKK^{mos}		212
Leishmania mexicana	MAPK	LMPK	lmMAPK^{lmpk}		370
Medicago sativa (alfalfa)	MAPK	MMK1, MsERK1, MsK7	msMAPK^{mmk1}		81, 168
		MMK2	msMAPK^{mmk2}		167
		MMK4	msMAPK^{mmk4}		166
Nicotiana tabacum (tobacco)	MKKK	NPK1	ntMK^{npk1}		12
	MKK	NPK2	ntMKK^{npk2}		311
	MAPK	Ntf3	ntMAPK^{ntf3}		372
		Ntf4	ntMAPK^{ntf4}		371
		Ntf6	ntMAPK^{ntf6}		371
		Wipkm, DS22	ntMAPK^{wipk}		310
		SIP kinase	ntMAPK^{sipk}		399
Petroselinum crispum (parsley)	MAPK	ERMK	pcMAPK^{ermk}		208
Petunia hybrida	MAPK	PMEK1	phMAPK^{mek1}		70
Pisum sativum (pea)	MAPK	D5, PsMAPK	psMAPK^{d5}		320

We have not included proteins whose sequences have not been published, even if they can be found in protein databases. as, Oat (*Avena sativa*); at, *Arabidopsis thaliana;* c, *Caenorhabditis elegans;* ca, *Candida albicans;* cca, carp (*Cyprinus carpio*); cco, quail (*Coturnix coturnix*); cd, hamster (*Cricetulus griseus*); d, *Drosophila melanogaster;* dd, *Dictyostelim discoideum;* eh, *Entamoeba histolytica;* fs, *Fusarium solani;* gg, chicken (*Gallus gallus*); h, human (*Homo sapiens*); lm, *Leishmania mexicana;* ms, alfalfa (*Medicago sativa*); nt, tobacco (*Nicotiana tabacum*); oc, rabbit (*oryctolagus cunniculus*); pc, parsley (*Petroselinum crispum*); ph, *Petunia hybrida;* ps, pea (*Pisum sativum*); r, rat (*Rattus norvegicus*). [a] hMKKK$^{tpl2}\alpha$ is the 58-kDa form, and hMKKK$^{tpl2}\beta$ is the 52-kDa form (9). [b] xMKK$^{mkk4}\alpha$ is the 446-amino acids form, and xMKK$^{mkk4}\beta$ is the 457-amino acids form (392). [c] hMKK$^{mek1}\alpha$ is the 394-amino acids form (402), and hMKK$^{mek1}\beta$ is the 367-amino acids form (309). [d] ccoMKKK$^{B\text{-}raf}$ α is the 767-amino acid form, and ccoMKKK$^{B\text{-}raf}$ β is the 807-amino acids form (91). References in higher eukaryotes (mammals, birds, amphibians, and fishes) section demonstrate that a given kinase is a MKKK, that is a kinase able to phosphorylate MKK. [e] YKL161c is a putative *S. cerevisiae* MKKK based on sequence homology (147). [f] Based on sequence homology, *C. elegans* locus CELC04G6 and CELR03G5 encode putative MAPK and MKKK, respectively (373). [g] hMAPK$^{p38\alpha r}\alpha$ corresponds to CSBP1 allele, hMAPK$^{p38\alpha r}\beta$ to CSBP2 allele, and hMAPK$^{p38\alpha r}\gamma$ to Mxi2 allele (197,397). [h] atMKKK$^{anp1}\alpha$ and atMKKK$^{anp1}\beta$ correspond to long splice variant (ANP1L) and to short splice variant (ANP1S), respectively (264).

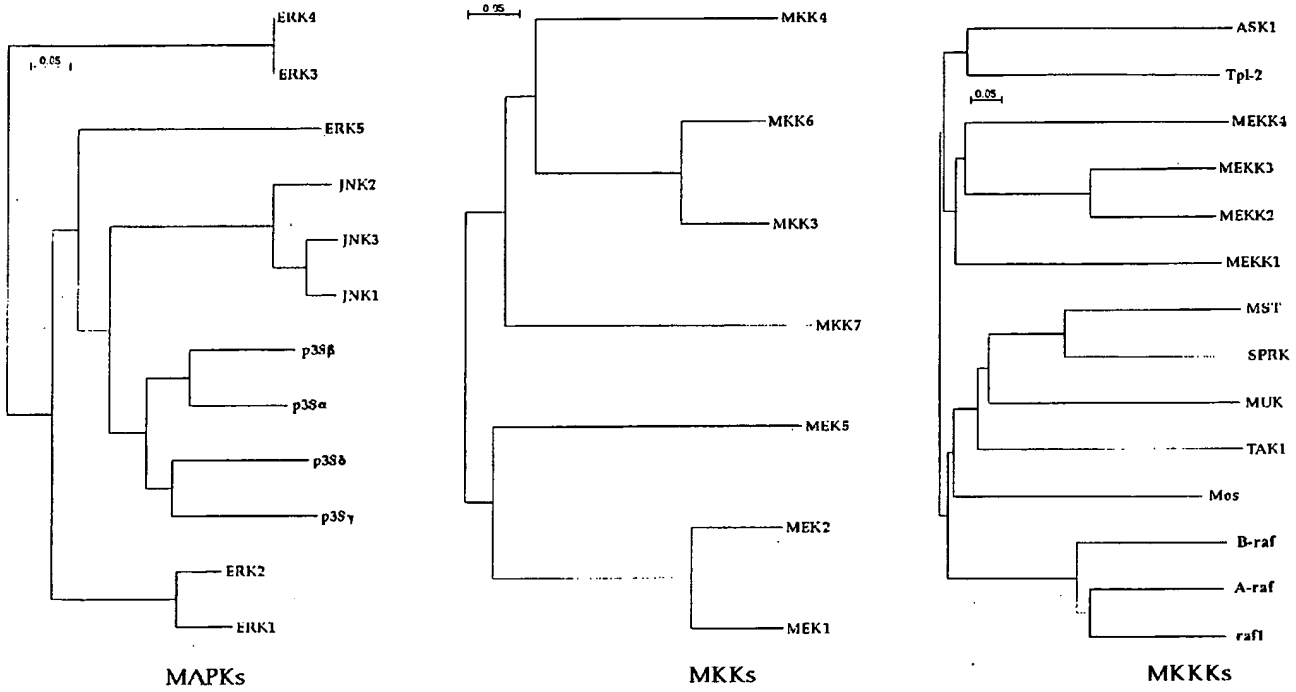


FIG. 2. Phylogenetic trees of mammalian MAPK, MKK, and MKKK family members. Dendrograms were created with CLUSTAL X program (334) using human sequences when available or mouse sequences otherwise (see Table 1).

Tyr regulation of MAPK activity has been proposed as why MAPK have never been identified as oncogenes (40), as could be expected from proteins regulating cell growth.

IV. YEAST MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS

Yeast probably represents the experimental model where the organization and regulation of MAPK pathways are best understood. Presently, five MAPK pathways have been well characterized in *S. cerevisiae*: the haploid mating pathway, invasive growth, cell wall remodeling, and two pathways involved in stress responses such as hyperosmolarity. These five MAPK pathways are discussed in detail in section IV. An important concept on MAPK pathways that has emerged from yeast studies is that the kinases employed in MAPK pathways are organized into modules. As discussed in section IV*A*, this is achieved by tethering to scaffold proteins as well as by direct interaction between the different kinases of the module. Organization into modules ensures segregation of the pathways from other signaling events in the cells and also allows the use of a given component kinase in more than one MAPK module without affecting the specificity of the response mediated by the MAPK pathways.

A. Organization of MAPK Modules

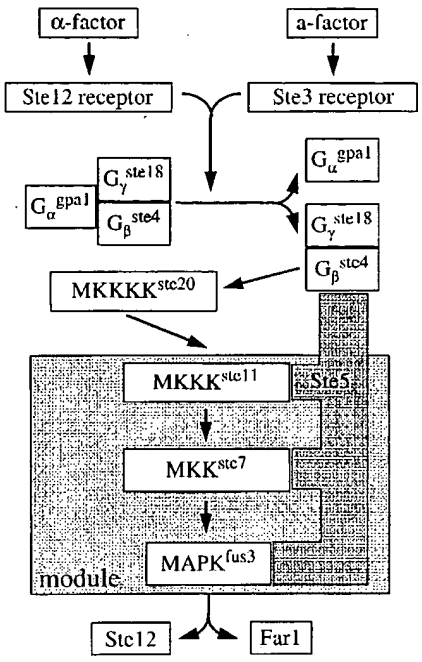
Genetic and biochemical studies in yeast have demonstrated that specific MKKK-MKK-MAPK signaling modules are functionally segregated from one another (98). In the budding yeast, *S. cerevisiae*, the three component MAPK modules are assembled either by tethering to scaffolding proteins such as Ste5 or by binding domains encoded in the kinases themselves. Ste5 is a 110-kDa dimeric protein that is capable of binding the three-kinase components of the pheromone-regulated mating MAPK pathway, $MKKK^{ste11}$, MKK^{ste7}, and $MAPK^{fus3}$. This ensures that this particular module is coordinately regulated and segregated from other signaling pathways. Tethering and segregation of MAPK modules may in fact be vital for MAPK pathways to function properly (217). Mitogen-activated protein kinase module kinases may have high affinity for each other in the absence of a tethering protein like Ste5. For example, $MKKK^{fus3}$ and MKK^{ste7} have an ~5 nM dissociation constant binding affinity, indicating they could have significant interaction in the the absence of Ste5 (13). Similarly, MKK^{pbs2} has the ability to bind several different MKKK involved in osmoregulation (see sect. IV*F*). The complexity of regulation of MAPK pathways is further exemplified by the fact that some kinases participate in more than one MAPK module. For example, $MKKK^{ste11}$ functions in the MAPK pathways for mating, pseudohyphal development, and osmoregulation. However, because of differential upstream inputs from MKKKK, GTP-binding proteins, and the assembly of three kinases into modules, there is generally little if any cross talk between the different MAPK pathways in *S. cerevisiae*.

B. Role of MAPK Modules in Yeast

Yeast may exist either as haploid or diploid cells. The haploid cells have two sexual phenotypes characterized by the expression of a set of genes involved in mating that are not expressed in diploids. The mating response to generate diploids is controlled by the a and α-pheromones that bind to their respective receptors that are coupled to a heterotrimeric G protein. Pheromone binding to its receptor leads to G protein activation and the dissociation of the $\beta\gamma$-subunit complex from α-GTP. In budding yeast, the $\beta\gamma$-complex binds to Ste5 and by a process still poorly understood stimulates activation of the mating MAPK pathway. In addition to mating, yeast respond to their environment with metabolic changes that involve MAPK pathways. For example, in response to starvation, yeast undergo dramatic morphological changes involving pseudohyphal formation and invasiveness in an attempt to find nutrients that are controlled in part by a MAPK pathway. Yeast adapt to additional adverse environmental conditions such as high or low osmolarity by activation of specific MAPK pathways. Thus many aspects of yeast physiology controlling their response to environmental cues are, at least in part, regulated by MAPK pathways (136). Knowledge of the entire genome sequence for the budding yeast *S. cerevisiae* has defined the number of protein kinases in the organism (147). Relative to MAPK modules, it appears that the *S. cerevisiae* genome encodes four MKKK, four MKK, and six MAPK (Table 1). Among the six different MAPK present in *S. cerevisiae* (147), only two cannot be attributed to one of the five well-characterized MAPK pathways: $MAPK^{smk1}$, which is involved in spore wall assembly, and the putative $MAPK^{YKL161C}$ identified by genome sequencing. $MAPK^{smk1}$ and $MAPK^{YKL161C}$ may be involved in as yet undefined MAPK pathways. $MAPK^{YKL161C}$ has a K-X-Y motif in its activation loop distinguishing it from other MAPK.

C. Mating Pathways in Haploid Yeast

The best-defined yeast MAPK pathway in *S. cerevisae* is involved in the mating of haploid cells (21, 58, 89, 305) (Fig. 3). The two haploid cell types (a and α) of *S. cerevisiae*, upon binding the sexual pheromone secreted by the opposite cell type (a-factor and α-factor), stop growing and differentiate into mating-competent cells by inducing transcription of mating genes. The *S. cerevisiae* genes whose disruption inhibited mating and caused sterility were designated as sterile (ste) genes. The seven transmembrane receptors for the α- and a-factors are designated as Ste2 and Ste3, respectively, and are coupled to a heterotrimeric G protein. Pheromone activation of the G protein induces the dissociation of the heterotrimeric G protein subunits designated Gpa1 (α-subunit), Ste4 (β-subunit), and Ste18 (γ-subunit). The released $\beta\gamma$-subunit



S. cerevisiae mating pathway

FIG. 3. *Saccharomyces cerevisiae* MAPK pathway used for mating. Note that Ste5 is a scaffolding protein that is able to bind to Ste4, $MKKK^{ste11}$, MKK^{ste7}, and $MAPK^{fus3}$.

complex (Ste4/Ste18) activates Ste20 and interacts with the scaffolding protein Ste5, resulting in the stimulation of the MAPK module $MKKK^{ste11}/MKK^{ste7}/MAPK^{fus3}$. It should be noted that involvement of Ste20 in the activation of the mating MAPK pathway is still debated, since it has not yet been unequivocally proven that Ste20 phosphorylates and activates $MKKK^{ste11}$. Activated $MAPK^{fus3}$ regulates the activity of transcription factors required for the expression of components of the mating pathway itself and genes necessary for cell cycle arrest and cell fusion (136).

It was initially thought that $MAPK^{fus3}$ and $MAPK^{kss1}$ were redundant kinases in the mating response, because genetic experiments indicated that expression of one protein could complement the mating defect induced by the absence of the other. This notion was widely accepted in the MAPK field even though a number of observations indicated that $MAPK^{fus3}$ and $MAPK^{kss1}$ were differently regulated and were in fact components of different signaling pathways. First, $MAPK^{fus3}$ and $MAPK^{kss1}$ are not activated by the same stimuli. $MAPK^{fus3}$ activity is very low, whereas $MAPK^{kss1}$ exhibits high activity, in the absence of mating pheromones. Conversely, in presence of pheromones, $MAPK^{fus3}$ is strongly activated but $MAPK^{kss1}$ only weakly if at all (13, 85). Second, $MAPK^{fus3}$ is expressed only in haploid cells, whereas $MAPK^{kss1}$ is expressed both in haploid and diploid cells, indicating that these two MAPK

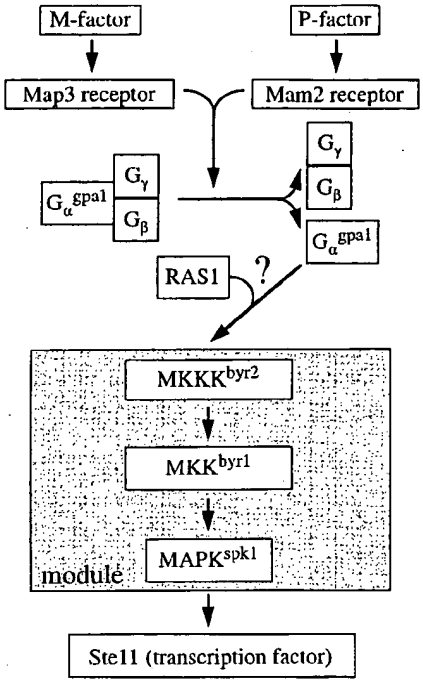
have different functions. Third, $MAPK^{fus3}$ and $MAPK^{kss1}$ have different substrate targets (259). With regard to substrate recognition, it has been shown that $MAPK^{fus3}$, but not $MAPK^{kss1}$, phosphorylates Far1 (85, 275) that represses the transcription of the G_1 cyclins Cln1 and Cln2 (83), leading to cell cycle arrest that is a feature associated with mating. Cumulatively, these studies suggested that $MAPK^{fus3}$ is the MAPK involved in mating, whereas $MAPK^{kss1}$ is not. This was confirmed recently with experiments showing that the expression of a kinase inactive mutant of $MAPK^{fus3}$ in a $MAPK^{fus3}$ null background hampered the ability of $MAPK^{kss1}$ to fulfill mating MAPK functions (217). The findings indicated that $MAPK^{kss1}$ cannot activate the mating MAPK pathway in wild-type cells because it is excluded from this pathway by $MAPK^{fus3}$. Only in null mutants lacking $MAPK^{fus3}$ can $MAPK^{kss1}$ substitute for $MAPK^{fus3}$ in the mating pathway, explaining the earlier results that suggested redundancy between the two MAPK. Thus $MAPK^{fus3}$ has an additional function that is to segregate the mating MAPK pathway from imposter proteins, in this case $MAPK^{kss1}$, that are normally involved in other signaling pathways. Segregation of the MAPK module involved in mating is further achieved by Ste5, the scaffold molecule which has no apparent enzymatic activity itself. Different domains of Ste5 bind, in the two-hybrid system, to $MKKK^{ste11}$ and $MAPK^{fus3}$ (53, 151, 184, 226, 280) as well as to Ste4 (G protein β-subunit) (365) and is predicted to couple the $\beta\gamma$-complex (Ste4/Ste18) to activation of the MAPK mating pathway (Fig. 3).

The NH_2-terminal portion of Ste5 (residues 177–229) contains a cysteine-rich region that is the prototype for the RING-H2 motif. Proteins of the zinc RING family possess two fingerlike domains connected by a linking region and require zinc for folding (18, 29). Crystal structures indicate that RING domains are globular pseudosymmetric folds that coordinate two zinc atoms through a cross-bridging element (14, 28). When this structure is mutated, the corresponding Ste5 mutants are unable to complement the mating defect of yeast strains lacking Ste5 (ste5Δ cells) (152). The RING-H2 mutants, while being able to bind $MKKK^{ste11}$, MKK^{ste7}, and $MAPK^{fus3}$ as efficiently as the wild-type protein, were not capable of binding Ste4. Moreover, the RING-H2 mutants could not dimerize (152). When the *Schistosoma japonicum* glutathione *S*-transferase protein, known to form stable dimers (230, 349), was fused to the COOH terminus of the RING-H2 Ste5 mutants, the resulting protein could complement the mating defect of ste5Δ cells but could not associate with Ste4. Oligomerization of Ste5 is, however, not sufficient to transduce the mating signal, since wild-type Ste5 fused to the *S. japonicum* glutathione *S*-transferase was able to complement ste5Δ cells but not ste4Δ ste5Δ cells (152). The conclusion of these studies is that the RING-H2 domain has inhibitory functions that can be alleviated by Ste4. Once the inhibitory role of the RING-H2 domain is suppressed, it oligomerizes, leading to the activation of the MAPK mating module, possibly by allowing Ste20 to phosphorylate $MKKK^{ste11}$. It is presently unclear if $MKKK^{ste11}$ phosphorylation is the primary regulatory event that controls its activity, even though it is a substrate for Ste20.

Stimulation of the MAPK mating pathway leads to the activation or repression of the activity of a variety of proteins. For example, Far1 phosphorylated by $MAPK^{fus3}$ binds to and inactivates the cell division control (Cdc)28/Cln kinase complex and thus inhibits cell growth. Phosphorylation of Far1 is critical, since a mutant allele of Far1 that is not fully phosphorylated is unable to mediate pheromone-induced cell cycle arrest (275). Ste12, a transcription factor mediating the induction of pheromone-response genes, is also phosphorylated by $MAPK^{fus3}$ (85). One of the functions of Ste12 is to induce Far1 transcription (266). Thus $MAPK^{fus3}$ regulates Far1 in two complementary ways: a direct phosphorylation-dependent activation and an increase in transcription through activation of Ste12.

Yeast cells that are ready to undergo mating must make sure that they do not grow, and this is achieved in part by the $MAPK^{fus3}$ phosphorylation and activation of Far1. Similarly, cells undergoing cell division should not be responsive to pheromones. Interestingly, the G1 cyclins that are inhibited by Far1 in mating competent cells are themselves inhibitors of the mating pathways in dividing cells. It has indeed been shown that Cln2 represses the mating MAPK pathway, and this inhibition takes place at the level of $MKKK^{ste11}$ (362). There are other examples demonstrating the reciprocal actions of the mating MAPK pathway and cyclin-dependent kinases. For example, the rat glucocorticoid receptor ectopically expressed in *S. cerevisiae* is phosphorylated by cyclin-dependent kinases and MAPK at distinct sites. Glucocorticoid receptor-dependent transcriptional enhancement is reduced in a yeast strain deficient in cyclin-dependent kinases but is increased in a strain devoid of $MAPK^{fus3}$ (186).

In the fission yeast *S. pombe*, mating involves a MAPK module containing homologs of the *S. cerevisiae* MAPK mating pathway ($MKKK^{byr2}$ is homologous to $MKKK^{ste11}$, MKK^{byr1} to MKK^{ste7}, and $MAPK^{spk1}$ to $MAPK^{fus3}$) (89) (Fig. 4). The *S. pombe* and *S. cerevisiae* mating pathways are controlled by different upstream regulators. First, in the budding yeast, the $G\beta\gamma$ complex transmits the signal, whereas in *S. pombe*, it is the $G\alpha$ subunit that transmits the signal. Second, although haploid budding yeast express the genes required for the pheromone response in all nutritional conditions, differentiation of fission yeast into mating competent cells is strictly dependent on nutritional starvation. The *S. pombe* Ras homolog Ras1 plays a role in the starvation-dependent control of the mating pathway, possibly in a $G\alpha$-independent manner (384). In response to mating pheromones, mutants defective in Ras1 or in Ste6, the fission yeast homolog of the Ras GDP/



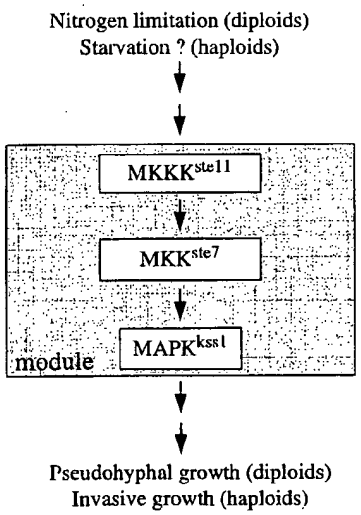
S. pombe mating pathway

FIG. 4. *Schizosaccharomyces pombe* MAPK pathway used for mating.

GTP exchange factor, are unable to induce transcription of the mat1-Pm gene that controls entry into meiosis (262). Ras1 may be directly linked to the *S. pombe* MAPK module involved in mating as suggested by the observation that Ras1 can bind MKKK^{byr2} in a two-hybrid assay (48, 344). Interestingly, a Ste5 equivalent has not been defined in *S. pombe*, nor is there evidence that phosphorylation of MKKK^{byr2} by a Ste20 homolog is required. Current evidence argues that GTP-binding proteins regulate MKKK^{byr2} activity.

D. Invasive Pathways: Pseudohyphal Pathway of Diploids and Invasive Growth of Haploids

In response to nitrogen starvation, diploid *S. cerevisiae* activate an intracellular pathway that will eventually lead to profound morphological changes. When starved for nitrogen, the elliptical diploid yeast undergo an asymmetric cell division to produce a long thin daughter cell that will keep producing long daughter cells. Because the mother and daughter cells remain attached, reiteration of this unipolar division pattern produces filaments composed of a linear chain of elongated cells called a pseudohypha. The mother yeast produce colonies on the surface of an agar plate, whereas the pseudohypha invades the agar (112, 113). Haploid cells can also invade the agar and grow beneath the surface. The response of haploids is not as dependent on nutritional starvation as the pseudohyphal development of diploid cells. In particular, nitrogen limitation seems to play no role in the invasive growth response of haploids (293). A MAPK module consisting of MKKK^{ste11}, MKK^{ste7}, and MAPK^{kss1} can transduce the signals leading to invasive growth (Fig. 5). Mitogen-activated protein kinase^{kss1} was initially thought to play no role in invasiveness because diploid mutants lacking MAPK^{kss1} had a normal pseudohyphal response (136). However, with the use of strains bearing hypermorphic alleles of MKKK^{ste11} and MKK^{ste7} to increase filamentous growth, it was shown that the absence of MAPK^{kss1} blocked hyperfilamentation, as well as the enhancement of the activity of promoters containing filamentation and invasion response elements. The kinase activity of MAPK^{kss1} is required for filamentous growth, both in haploids and diploids, because removal of MAPKK^{ste7}, the upstream regulator of MAPK^{kss1}, or point mutations impairing the catalytic activity of MAPK^{kss1} result in severe filamentation defects (121, 217). If MAPK^{kss1} is required for a normal invasive response, how is it that MAPK^{kss1} knockout mutants have no pseudohyphal defect? The postulated answer is that MAPK^{kss1} also has an inhibitory function in the invasive response and that there are MAPK-independent signaling pathways mediating invasiveness (121, 217). In the absence of MAPK^{kss1}, the MAPK-independent pathways can stimulate the invasive growth because they are not hampered by the inhibitory function of MAPK^{kss1}. It has been possible to genetically separate the inhibitory and stimulatory functions of MAPK^{kss1} on pseudohyphal development. There are mutants of



The pseudohyphal pathway of diploids and the invasive growth response of haploids

FIG. 5. *Saccharomyces cerevisiae* MAPK pathway used during invasive responses.

$MAPK^{kss1}$ that have lost their inhibitory function that when expressed lead to hyperfilamentation in a $MAPK^{kss1}$ null background (217). In contrast, in the situation where $MAPK^{kss1}$ cannot be activated (i.e., when $MAPKK^{ste7}$ is absent), filamentation is drastically reduced because $MAPK^{kss1}$, while unable to activate filamentation since this requires the stimulation of its catalytic activity, retains its negative function (121, 217). This also shows that the inhibitory function of $MAPK^{kss1}$ does not require its catalytic activity. When not activated by MKK^{ste7}, $MAPK^{kss1}$ is thus an inhibitor or silencer of the invasive growth response. In contrast, when $MAPK^{kss1}$ is activated by MKK^{ste7}, it stimulates invasive growth, and this response depends on an intact kinase activity. The negative function of $MAPK^{kss1}$ is mediated by its interaction with its target Ste12. $MAPK^{kss1}$ interacts with Ste12 predominantly in its inactive state. This interaction inhibits the activity of Ste12. Once $MAPK^{kss1}$ is activated, it no longer binds and inhibits Ste12; rather, active $MAPK^{kss1}$ phosphorylates and activates Ste12, leading to activation of genes under the control of promoters containing filamentation and invasion response elements (217).

Invasiveness is more pronounced in the absence of $MAPK^{fus3}$ (either in mutant haploids cells or in diploids that do not express $MAPK^{fus3}$) (121, 293), indicating that $MAPK^{fus3}$ functions as a repressor of the invasive growth response. Thus, when activated, $MAPK^{fus3}$ and $MAPK^{kss1}$ have opposite functions in invasive growth. Possibly, one role of the MAPK pathway employing $MAPK^{fus3}$ is to suppress invasive growth in haploid cells to facilitate their entry into the mating program where the cells must stop growing. In such a scenario, in the presence of pheromones, the inhibitory activity of $MAPK^{fus3}$ on invasive growth would be greater than the stimulatory activity of $MAPK^{kss1}$. This is consistent with the fact that pheromones activate $MAPK^{fus3}$ much more strongly than $MAPK^{kss1}$ (13, 85). Clearly, the balance of these kinase activities, both regulated by MKK^{ste7}, will play a role in determining whether a cell will exhibit an invasive response.

The exact role of the $MKKK^{ste11}/MKK^{ste7}/MAPK^{kss1}$ module in the control of invasive growth is still incompletely understood. The need for a better understanding of invasive growth becomes obvious when it is considered that removal of all the components of the $MKKK^{ste11}/MKK^{ste7}/MAPK^{kss1}$ module does not markedly affect the invasive growth response (121). Thus, even though genetic studies show invasive growth can be strongly affected by mutants in the $MKKK^{ste11}/MKK^{ste7}/MAPK^{kss1}$ module, the pathway itself is not required. This shows that filamentation and invasion response elements are under the control of alternative regulatory pathways in the absence of a functional $MKKK^{ste11}/MKK^{ste7}/MAPK^{kss1}$ pathway. Such pathways may involve Ras, since it has been shown that activated forms of Ras potentitate the invasive growth response (113, 121, 250).

E. Cell Wall Remodeling Pathway

In yeast, growth depends on efficient cell wall remodeling, and this response also uses a MAPK pathway (Fig. 6). In the budding yeast, the MAPK module is composed of $MKKK^{bck1}$, MKK^{mkk1} or MKK^{mkk2}, and $MAPK^{mpk1}$. The biological relevance of having two seemingly redundant MKK in the cell wall remodeling pathway is unclear. The upstream regulator of the MAPK module of the cell wall remodeling pathway is the yeast homolog of mammalian protein kinase C (PKC), PKC1, which could function as a MKKKK for this pathway although it has not been proven biochemically that PKC1 can directly phosphorylate and activate $MKKK^{bck1}$. Mutants lacking PKC1, $MKKK^{bck1}$, or $MAPK^{mpk1}$ lyse under isotonic conditions because of a deficiency in cell wall construction (154, 199, 206). However, the phenotypes of the mutants defective in PKC1 are more severe than in the mutants defective in the downstream components of the pathway; PKC1 mutants have a lysis defect at all temperatures (200). This suggests that PKC1 controls two pathways required for an optimal cell-wall remodeling response, one of which contains the $MKKK^{bck1}/MKK^{mkk1}$ or $MKK^{mkk2}/MAPK^{mpk1}$ module.

In the fission yeast *S. pombe*, $MAPK^{pmk1}$ may be part of a MAPK pathway involved in maintenance of cell wall integrity (337, 395). However, $MAPK^{pmk1}$ may not be a downstream target of PKC in *S. pombe* but may function in coordination with PKC to regulate cell integrity (337). The upstream regulators of $MAPK^{pmk1}$ remain to be characterized in fission yeast.

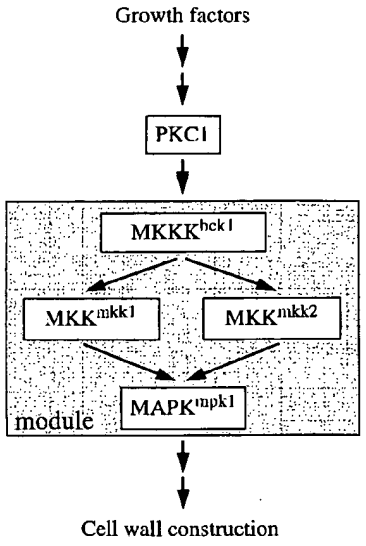


FIG. 6. *Saccharomyces cerevisiae* MAPK pathway used for cell wall construction. PKC, protein kinase C.

F. Osmosensor and Stress Pathways

The budding yeast *S. cerevisiae* activates two pathways in response to hyperosmolarity (220) (Figs. 7 and 8). The osmosensors that stimulate these pathways function in a very different manner. One pathway is regulated by a set of three proteins functioning like the prokaryotic two-component transduction system, and the other osmosensor is an integral membrane protein. These two sensors regulate two different MAPK pathways utilizing common kinase elements that will lead to the transcription of genes necessary for survival in hyperosmotic conditions such as those required for the synthesis of glycerol to increase the internal osmolarity (136). Although external high osmolarity will activate both of these MAPK pathways, the corresponding osmosensors do not detect the same osmolarity changes. The integral membrane osmosensor is activated by high osmolarity and stimulates its corresponding MAPK pathway. In contrast, the two-component osmosensor is active in low-osmolarity conditions, and this results in the repression of the associated MAPK module. In high-osmolarity conditions, however, this osmosensor is inactivated and no longer inhibits the activation of the MAPK. Apparently, fission yeast also employ

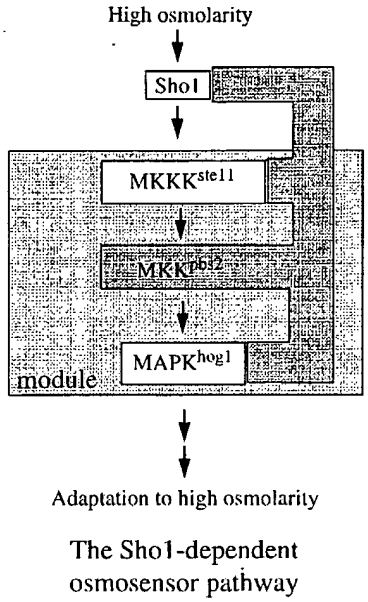


FIG. 8. Sho1-dependent osmosensor MAPK pathway in *Saccharomyces cerevisiae*. MKK^{pbs2} functions both as a MKK and as a scaffold protein that binds to Sho1, MKK^{ste11}, and MAPK^{hog1}.

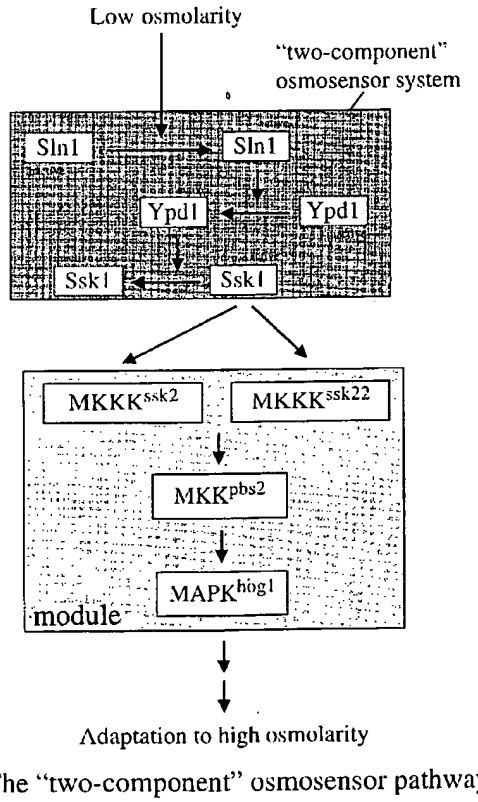


FIG. 7. "Two-component" osmosensor MAPK pathway of *Saccharomyces cerevisiae*. Solid and dotted boxes correspond to active and inactive state of proteins, respectively.

two related MAPK pathways to sense changes in osmolarity. Two different MAPK pathways are thus activated in response to hyperosmotic conditions in both *S. cerevisiae* and *S. pombe*. The reason for this is not yet understood, but it is likely that each of the osmosensor MAPK pathways do not fulfill exactly the same function in response to increased osmolarity and that they may be differentially involved in sensing and responding to other types of stress.

1. "Two-component" osmosensor pathway

Two-component transduction systems are commonly found in prokaryotes. A two-component system is composed of a sensor molecule and a response-regulator molecule. Typically, a sensor protein has an extracellular input domain and a cytoplasmic histidine kinase domain. A typical response-regulator is a cytosolic protein containing the receiver domain and a DNA binding domain. When the sensor protein is activated, it phosphorylates a histidine residue within its kinase domain and transfers this phosphate group to an aspartic acid in the receiver domain of the cognate response-regulator molecule, resulting in the switching of its output function that is generally transcriptional activation. It is because the signaling pathway is composed of only two proteins that it is called a two-component system. The two-component osmosensor in yeast is, however, composed of three proteins, Sln1, Ypd1, and Ssk1, that funtionally behave as two linked two-component systems (Fig. 7). Sln1 corresponds to the first two-component system, since it contains both

a histidine kinase domain and a receiver domain. The Ypd1-Ssk1 pair functions as the second two-component system. Ypd1 is phosphorylated on a histidine residue as a result of a transfer of the phosphate on the aspartic acid of Sln1. This phosphate is then transferred to Ssk1 on an aspartic acid (278). Sln1, Ypd1, and Ssk1 thus function as a multistep phosphorelay. The advantage of such a system may be its ability to be regulated at different levels. Ssk1 has a kinase activity and is able to stimulate a MAPK module composed of $MAPK^{hog1}$, MKK^{pbs2}, and two apparently redundant MKKK, $MKKK^{ssk2}$ and $MKKK^{ssk22}$ (23, 35, 220) (Fig. 7). Ssk1 is inactive when phosphorylated (i.e., in low-osmolarity conditions) and activated by high osmolarity (when the sensor Sln1 is inactive), which eventually leads to stimulation of $MAPK^{hog1}$ and transcription of genes necessary for survival in hyperosmotic conditions.

2. *Sho1-dependent osmosensor pathway*

In addition to the Sln1-Ypd1-Ssk1 osmosensor pathway, the budding yeast has an alternate way of sensing hyperosmolarity that relies on the Sho1 osmosensor (220) (Fig. 8). Sho1 contains four transmembrane domains and a COOH-terminal cytoplasmic region with a Src homology 3 (SH3) domain (220). In high-osmolarity conditions, $MKKK^{ste11}$ is activated in a Sho1-dependent manner. $MKKK^{ste11}$ then phosphorylates and activates MKK^{pbs2}, which activates $MAPK^{hog1}$ in the module (277). Activated $MAPK^{hog1}$ regulates the transcription of genes required for survival in hyperosmotic conditions (Fig. 8). In addition to its roles in the mating and the pseudohyphal pathways, $MKKK^{ste11}$ is also involved in an osmoregulation pathway, indicating that $MKKK^{ste11}$ is a component of at least three different MAPK modules. Activation of $MKKK^{ste11}$ in one module does not necessarily imply that $MKKK^{ste11}$ present in the other MAPK modules will be activated. The reason for this is the segregation and assembly of different MAPK modules regulated by different upstream inputs. For example, pheromones induce the phosphorylation and activation of $MAPK^{fus3}$ but not $MAPK^{hog1}$, even though $MKKK^{ste11}$ is the upstream regulator of both MAPK, indicating that $MKKK^{ste11}$ is activated in the mating pathway but not in the osmosensor pathway. Conversely, hyperosmolarity will activate $MKKK^{ste11}$ in the Sho1-dependent osmosensor pathway as assessed by the fact that $MAPK^{hog1}$ gets phosphorylated, but hyperosmolarity does not lead to the activation of $MKKK^{ste11}$ in the mating pathway demonstrated by the fact that $MAPK^{fus3}$ is not phosphorylated (277).

The basis for the differential control is accounted for by the regulation of the mating MAPK module by Ste5 and the $G\beta\gamma$ subunits (Ste4/Ste18) while MKK^{pbs2} is able to bind to Sho1, $MKKK^{ste11}$, and $MAPK^{hog1}$ [the interaction of Sho1 and MKK^{pbs2} being mediated by the poly-proline-rich region of MKK^{pbs2} and the SH3 domain of Sho1 (220)]. Thus MKK^{pbs2} also functions as a scaffold protein to segregate the $MKKK^{ste11}/MKK^{pbs2}/MAPK^{p38}$ module for regulation by Sho1 (220, 277). The properties of Ste5 and $MAPKK^{pbs2}$ in the mating and Sho1-dependent MAPK pathways, respectively, ensure that the two pathways are segregated from one another.

3. *Osmosensing pathways in S. pombe*

In fission yeast, MAPK pathways are also activated under stress conditions to mediate survival responses. Similar to budding yeast, it appears that two related MAPK pathways are activated in response to environmental stress. One is composed of the $MKKK^{wik1}/MKK^{wis1}/MAPK^{spc1}$ module, and the other is most likely composed of the $MKKK^{win1}/MKK^{wis1}/MAPK^{spc1}$ module (299, 312, 313) (Fig. 9). The pathway using $MKKK^{win1}$ may be predominant in conditions of osmotic stress because $MKKK^{wik1}$ is not essential for osmosignaling (299). Interestingly, activation of $MAPK^{spc1}$ can be achieved in the absence of MKK^{wis1} phosphorylation in response to heat shock and oxidative stress. The inactivation of the Pyp1 protein tyrosine phosphatase is likely to be involved in this alternative $MAPK^{spc1}$ activation mechanism (299). The upstream regulator of $MKKK^{wik1}$, Mcs4, is the homolog of the *S. cerevisiae* Ssk1 protein (312). Thus, in lower eukaryotes, the stress-activated MAPK pathway appears to be controlled by a conserved two-component system. In addition to involvement in stress responses, MKK^{wis1}, $MAPK^{spc1}$, and Pyp1 have roles in cell cycle progression. MKK^{wis1} was originally isolated as a mitotic inducer (360), and Pyp1 has been shown to negatively regulate entry into mitosis (236).

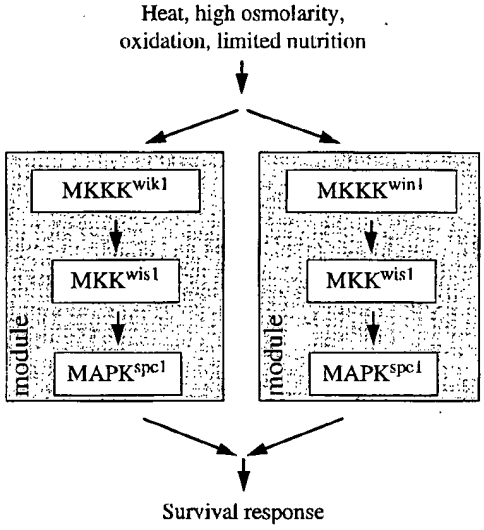


FIG. 9. Stress-induced MAPK pathways in *Schizosaccharomyces pombe*.

G. Sporulation Pathway

Yeast sporulation is the process, involving meiosis, that leads to the packaging of haploid nuclei into spores. This reponse occurs only in diploids and is elicited by nutritional starvation (241). Upon completion of meiosis, the four-haploid nuclei, which still remain within a single nuclear membrane, are enveloped by the double membraneous prospore wall. The spore wall is then deposited from the space between the layers of the prospore wall. The final differentiated spore wall consists of four layers. The two inner layers appear indistinguishable from the vegetative cell wall, whereas the third layer is a spore-specific structure composed primarily of chitosan and chitin. The outermost electron-dense layer is a dityrosine coat (36, 37). One gene encoding a MAPK, $MAPK^{smk1}$, has been shown to be involved in the sporulation pathway ($MAPK^{smk1}$ mutants are defective in spore wall assembly) (185). A putative MAPK module, which employs $MAPK^{smk1}$ and an uncharacterized MKKK and MKK, may thus be used to regulate the sporulation response. A MKKKK homolog (Sps1) has also been shown to be involved in the sporulation pathway. Both Sps1 and $MAPK^{smk1}$ mutants display similar phenotypes: they both proceed normally through meiosis but then are defective in spore wall assembly (136, 185). This suggests that Sps1 controls the putative MAPK module mediating the sporulation response.

V. MAMMALIAN MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS

One important point that emerges from the the studies of the yeast MAPK pathways is that MAPK pathways form modules that are held together through protein-protein interactions. Activation of one MAPK pathway does not normally lead to the activation of other MAPK pathways, even if a given component is found within multiple pathways. Mitogen-activated protein kinase pathways are thus spatially regulated in yeast. It is thus predicted that this also applies to the mammalian MAPK pathways. In mammalian cells, however, multiple MAPK pathways can be activated by a single receptor type. For example, the high-affinity FcεR1 receptor for IgE activates the $MAPK^{erk}$, $MAPK^{jnk}$, and $MAPK^{p38}$ pathways in mast cells. Mammalian cells also do not lend themselves to genetic studies as performed in yeast. For this reason, many studies in mammalian cells involve overexpression of activated and inhibitory mutant components of MAPK pathways. A potential pitfall, however, is that this methodology may perturb the segregation mechanisms holding individual MAPK modules. The challenge in the forthcoming years will be to improve existing methods or develop new technologies in mammalian systems to overcome these limitations. Obviously, gene knock-out strategies may be particularly usefull in this context.

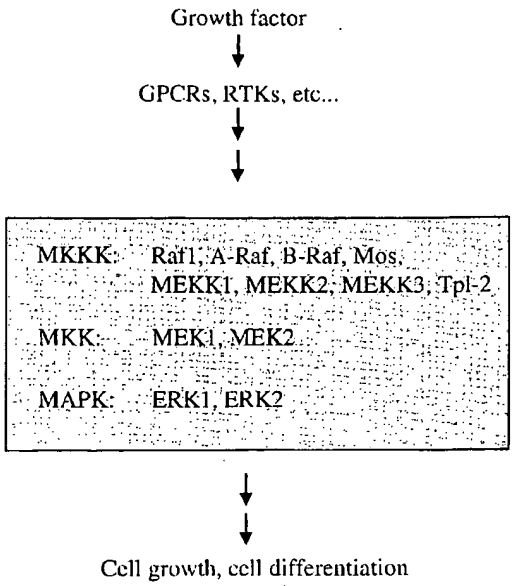


FIG. 10. MKKK, MKK, and MAPK that can be components of $MAPK^{erk}$ pathway. GPCR, G protein-coupled receptor; RTK, related tyrosine kinase; ERK, extracellular-regulated kinase; MEK, MAPK or ERK kinase.

In yeast, it appears that transcription factors comprise the majority of the known substrates regulated by MAPK pathways. In mammalian cells, many MAPK substrates defined to date are also transcription factors. In addition, several cytoskeletal proteins, protein kinases, and phopholipases are also substrates for specific MAPK. As our understanding of the yeast and mammalian MAPK pathways increases, it is likely that additional classes of MAPK substrates will be defined.

Four types of MAPK pathways have been defined to date in mammalian cells. However, within a given pathway, several MKKK, MKK, and MAPK can generally be found to be interchangeable. For example, there are 3 isoforms and 10 different splice variants of MAPK in the c-Jun kinase ($MAPK^{jnk}$) pathway (123). The relevance of this complexity is poorly understood and is difficult to assess, because it is not straightforward to dissect the role of individual components of the MAPK pathways in mammalian cells. Mammalian MAPK pathways are involved in a diverse set of responses affecting cell fate, including cell proliferation and differentiation, adaptation to environmental stress, and apoptosis.

A. $MAPK^{erk}$ Pathway

The best described MAPK signaling pathway in mammalian cells is the extracellular signal-regulated kinase

($MAPK^{erk}$) pathway. This pathway can apparently include a number of different MKKK and MKK (Fig. 10). There are five MAPK defined as ERK ($MAPK^{erk1-5}$) (52, 274, 308). However, amino acid sequence comparisons indicate that these proteins belong to different subfamilies of MAPK (see Fig. 2). There is actually more divergence between the $MAPK^{erk1}$/$MAPK^{erk2}$ subfamily and the $MAPK^{erk3}$/$MAPK^{erk4}$ subfamily than between the $MAPK^{erk1}$/$MAPK^{erk2}$ subfamily and any other MAPK. Of the collective group of MAPK referred to as ERK, $MAPK^{erk1}$ and $MAPK^{erk2}$ are the most extensively studied. $MAPK^{erk1}$ and $MAPK^{erk2}$ are 44- and 42-kDa isoforms, respectively. $MAPK^{erk1/2}$ is activated by many different inputs to the cell that lead to the activation of several transcription factors and other serine threonine kinases contributing to cellular proliferation, differentiation, cell cycle regulation, and cell survival. The other ERK are less well characterized. $MAPK^{erk3}$ is localized in the nucleus and is activated by PKC isoforms (52, 302). $MAPK^{erk4}$ has been shown to be activated in response to nerve growth factor (NGF) and EGF via a Ras-dependent pathway (274). The MAPK pathway employing $MAPK^{erk3}$ and $MAPK^{erk4}$ remains to be characterized. $MAPK^{erk5}$ is discussed in section v*D*.

1. $MAPK^{erk1/2}$

For further discussion in this section, we focus on the regulation of $MAPK^{erk1}$ and $MAPK^{erk2}$, which will be collectively referred to as $MAPK^{erk1/2}$. Upon activation, $MAPK^{erk1/2}$ phosphorylate substrate proteins on serine or threonine residues within a proline-directed motif. Pro-Leu-Ser/Thr-Pro is the most stringent consensus sequence for substrate recognition by $MAPK^{erk1/2}$ (42). Several cytoplasmic proteins have been shown to be substrates for $MAPK^{erk1/2}$. Proteins known to be phosphorylated by $MAPK^{erk1/2}$ include the S6 kinase $p90^{rsk}$, cytosolic phospholipase A_2, and the juxtamembrane region of the EGF receptor (210, 308, 366). Several microtubule-associated proteins (MAP) are also substrates for $MAPK^{erk1/2}$, including MAP-1, MAP-2, MAP-4, and Tau (308).

$MAPK^{erk1/2}$ phosphorylation of $p90^{rsk}$ at threonine-79 and threonine-396 contributes to activation of the kinase (261, 308). One consequence of $p90^{rsk}$ activation is its translocation to the nucleus, where it phosphorylates c-Fos (308). $p90^{rsk}$-induced phosphorylation of c-Fos occurs at serine-362 in the COOH-terminal transrepression domain. $p90^{rsk}$ also phosphorylates glycogen synthase kinase 3 (GSK3) at serine-9 near the NH_2 terminus, resulting in the inhibition of GSK3 kinase activity. Glycogen synthase kinase 3 has been shown to negatively regulate c-Jun. Thus stimulation of $p90^{rsk}$ by $MAPK^{erk1/2}$ results in the regulation of both c-Fos and c-Jun. The transient inactivation of GSK3 has been proposed to enable the rapid activation of c-Jun and possibly AP-1 activity (82, 308). The regulation of additional kinases by $MAPK^{erk1/2}$ can, therefore, result in amplification of the signaling downstream of $MAPK^{erk1/2}$.

The regulation of cytosolic phospholipase A_2 also generates active signaling molecules for the regulation of cellular physiology. Cytosolic phospholipase A_2 is phosphorylated at serine-505 by $MAPK^{erk1/2}$ (119). This phosphorylation event activates the enzyme that catalyzes the release of arachidonic acid. This is the rate-limiting step in the biosynthesis of eicosanoids (i.e., prostaglandins, leukotrienes). These compounds are important regulators of many physiological responses in cells (210, 366).

$MAPK^{erk1/2}$ is also able to phopshorylate the EGF receptor, the Ras exchange factor Sos, $MKKK^{raf1}$, and MKK^{mek1}. The phosphorylation of each of these proteins by $MAPK^{erk1/2}$ is believed to reduce their catalytic activity. Each of these proteins is involved in a signal pathway involving $MAPK^{erk1/2}$, and their phosphorylation would provide a feedback mechanism for controlling the activity of upstream regulators of the $MAPK^{erk1/2}$ pathway (366).

In addition to phosphorylating cytoplasmic proteins, activated $MAPK^{erk1/2}$ is translocated to the nucleus and phosphorylates several different transcription factors. Transcription factors phosphorylated and activated by $MAPK^{erk1/2}$ include Elk1, Ets1, Sap1a, c-Myc, Tal, and signal transducer and activator of transcription (STAT) proteins; Myb activity is believed to be inhibited by $MAPK^{erk1/2}$ (159, 203, 308, 366, 375).

2. *MKKK in the* $MAPK^{erk1/2}$ *pathway*

Ligation of many receptors leads to the activation of $MAPK^{erk1/2}$ through the activation of Ras. This was inferred from the observation that Ras activates $MKKK^{raf1}$ and that activated $MKKK^{raf1}$ is sufficient to stimulate the $MAPK^{erk1/2}$ signaling pathway (225, 319). Ras in the GTP-bound form binds to the NH_2-terminal regulatory domain of $MKKK^{raf1}$ (179). The NH_2-terminal regulatory domain, by itself, of $MKKK^{raf1}$ has high affinity for Ras · GTP and functions as an effective dominant negative mutant. Although $MKKK^{raf1}$ interacts with GTP-bound Ras, it is unclear whether the Ras · GTP interaction with $MKKK^{raf1}$ is sufficient to activate $MKKK^{raf1}$ (400). One function of Ras · GTP is to localize $MKKK^{raf1}$ to the plasma membrane where it becomes activated (80, 225). Support for this hypothesis was demonstrated by the addition of the Ras prenylation sequence (CAAX box) to the COOH terminus of $MKKK^{raf1}$ (131). The prenylated plasma membrane targeted $MKKK^{raf1}$-CAAX chimera has seven times greater kinase activity at similar levels of expression relative to wild-type $MKKK^{raf1}$ (201). $MKKK^{raf1}$-CAAX is constitutively activated and is oncogenic in fibroblast transformation assays. Once $MKKK^{raf1}$ is at the plasma membrane, it becomes tyrosine phosphorylated on Tyr-340 and Tyr-341 by membrane-bound tyrosine kinases including c-Src (223, 225). Treatment of $MKKK^{raf1}$ with tyrosine phosphatases inactivates $MKKK^{raf1}$, further supporting the importance of tyrosine phosphorylation for $MKKK^{raf1}$ activation (72). However, the role that tyrosine phosphorylation plays in $MKKK^{raf1}$

activation remains controversial, since mutation of the tyrosines on $MKKK^{raf1}$ reduces but does not eliminate its kinase activity upon activation of Ras (223, 225). A closely related homolog of $MKKK^{raf1}$, $MKKK^{B\text{-}raf}$ does not have the tyrosines at positions 340 and 341 but is activated by Ras, suggesting that tyrosine phosphorylation is not obligatory for activation of all $MKKK^{raf}$ family members (225). In addition to tyrosine phosphorylation, $MKKK^{raf1}$ is phosphorylated on serine residues 43, 259, 499, and 621 and is phosphorylated on threonine residue 268 (180, 225). Because treatment of cultured cells with phorbol esters leads to $MKKK^{raf1}$ activation and a strong $MAPK^{erk1/2}$ activation (144), it is reasonable to suspect that PKC isoforms that are activated directly by phorbol esters phosphorylate $MKKK^{raf1}$ leading to its activation. Indeed, coexpression of PKC-β with $MKKK^{raf1}$ stimulates $MKKK^{raf1}$ activation in a phorbol ester-dependent manner (227). Furthermore, PKC can also phosphorylate Ser-259 and Ser-499 of $MKKK^{raf1}$ in vitro (180), suggesting that tyrosine and serine phosphorylation of $MKKK^{raf1}$ controls its kinase activity when it is at the plasma membrane.

Recently, it was shown that $MKKK^{raf1}$ must be associated with Ras · GTP for its activation by PKC (222). Phorbol esters stimulate GTP binding to Ras, but the activation of $MKKK^{raf1}$ by PKC was not blocked by dominant negative Ras (N17Ras). N17Ras, which interacts with the exchange factor Sos, will block the activation of $MKKK^{raf1}$ by receptor tyrosine kinases, suggesting that PKC activates Ras by a mechanism involving a Ras exchange factor different from the one used by tyrosine kinases.

Proteins in addition to Ras bind and regulate $MKKK^{raf1}$ activity. The 14–3-3 proteins were initially discovered to interact with $MKKK^{raf1}$ in two-hybrid screening (101, 103). The 14–3-3 proteins have a variety of biological properties and recently have been shown to be involved in cell cycle control in both yeast and mammalian cells (5, 94, 135, 225, 291). The 14–3-3 proteins are dimers that may function as scaffolds or anchors to localize signaling proteins including protein kinases such as $MKKK^{raf1}$. The 14–3-3 proteins also bind to motifs in proteins that frequently include phosphoserine and phosphothreonine residues (255, 386). Binding of 14–3-3 protein to phosphoserine/threonine motifs in proteins has been demonstrated to protect these sites from dephosphorylation by phosphatases (56). Association of 14–3-3 proteins with $MKKK^{raf1}$ may prevent $MKKK^{raf1}$ inactivation by dephosphorylation and prolong its activation (225). Both 14–3-3β and 14–3-3ϵ associate with the NH_2-terminal regulatory domain of $MKKK^{raf1}$ (99). Mutation of Cys-162, Cys-168 in the cysteine-rich domain or Ser-259 of $MKKK^{raf1}$ prevents the association of 14–3-3 isoforms with $MKKK^{raf1}$. Mutation of Cys-162 or Cys-168 to serines has also been reported to block the activation of $MKKK^{raf1}$ by oncogenic Ras (225), but this finding has not been repeated by other investigators (95, 234). Despite the clear interaction of 14–3-3 proteins with MKK^{raf1}, it is still not apparent what the exact function of this interaction is with regard to regulation. Different investigators have found that 14–3-3 binding to $MKKK^{raf1}$ enhances, suppresses, or has no effect on $MKKK^{raf1}$ kinase activity (95, 101, 153, 234). It seems most likely that 14–3-3 proteins will be found to function primarily in organizing signal transduction systems such as upstream regulators of MAPK modules by controlling the proximity of these proteins with different MKKK (94).

$MKKK^{raf1}$ can also be phosphorylated by $MAPK^{erk1/2}$ and PKA (cAMP-dependent protein kinase) (225, 240, 308, 348). These phosphorylations inhibit $MKKK^{raf1}$ activity. The $MAPK^{erk1/2}$-mediated inhibition of $MKKK^{raf1}$ may be a mechanism to limit the extent of the activation of the pathway via a classical feedback inhibition mechanism. The inhibition of $MKKK^{raf1}$ signaling by PKA is a mechanism whereby receptor systems that regulate cAMP synthesis can negatively regulate the $MAPK^{erk1/2}$ pathway, allowing integration and control of these pathways by multiple inputs, both positive and negative.

Recently, MKK^{mek1}, the downstream effector of $MKKK^{raf1}$ in the $MAPK^{erk1/2}$ pathway, has been shown to increase the activity of $MKKK^{raf1}$ in a Ras- and Src-independent manner (408). The signal transmitted through the $MAPK^{erk1/2}$ pathway may thus be amplified when it reaches the MKK^{mek1} level, but it is negatively regulated when $MAPK^{erk1/2}$ is activated. This type of dual regulation may be important to determine the duration and strength of the $MAPK^{erk1/2}$ response.

There are two additional $MKKK^{raf}$ members, $MKKK^{A\text{-}raf}$ and $MKKK^{B\text{-}raf}$, whose pattern of expression is more restricted than that of $MKKK^{raf1}$ (324). The large 96-kDa form of $MKKK^{B\text{-}raf}$ is expressed in many neuronal and neuroendocrine cell types and appears to be the major MKK^{mek} activator in brain (43, 388). A smaller 68-kDa splice variant is expressed in fibroblasts and many other cell types. $MKKK^{B\text{-}raf}$ is also activated in NGF- and EGF-stimulated PC-12 cells (158). $MKKK^{A\text{-}raf}$ activates MKK^{mek} in cardiac myocytes after endothelin-1 stimulation (22). However, although $MKKK^{B\text{-}raf}$ is strongly activated by oncogenic Ras alone, maximal activation of both $MKKK^{raf\text{-}1}$ and $MKKK^{A\text{-}raf}$ requires additional inputs (224). Such inputs could involve upstream kinases such as Src, PKC, other MKKKK, or additional protein interactions such as with 14–3-3 proteins. A glimpse of such differential regulation has recently been discovered with $MKKK^{B\text{-}raf}$. The 96-kDa $MKKK^{B\text{-}raf}$ protein can be activated by a second GTP-binding protein, Rap1. Rap1 is phosphorylated near its COOH terminus by PKA (204). Treament of cells with cAMP leads to phosphorylation of Rap1, which enhances exchange of GDP for GTP. Rap1 · GTP binds and activates $MKKK^{B\text{-}raf}$ in a manner similar to Ras activation of $MKKK^{raf1}$ (348). Rap1 does not activate $MKKK^{raf1}$. The 68-kDa form of $MKKK^{B\text{-}raf}$ neither interacts with nor is regulated by Rap1. Rap1 appears to be expressed in most if

not all cells. Thus, in cells expressing the 96-kDa form of $MKKK^{B\text{-}raf}$, cAMP activation of PKA will stimulate the $MKKK^{B\text{-}raf}/MKK^{mek}/MAPK^{erk1/2}$ module while inhibiting $MKKK^{raf\text{-}1}$.

3. MKK in the $MAPK^{erk1/2}$ pathway

MKK^{mek1} and MKK^{mek2} are the MKK in the three-kinase component $MAPK^{erk1/2}$ activation modules (225, 366). MKK^{mek1} and MKK^{mek2} are highly homologous in primary amino acid sequence and function as dual threonine/tyrosine kinases. $MAPK^{erk1/2}$ have a Thr-Glu-Tyr sequence in the activation loop of the kinase catalytic domain that is phosphorylated by activated $MKK^{mek1/2}$ (366). MKK^{mek1} contains a nuclear export signal that excludes it from the nucleus (104). It has been proposed that MKK^{mek1} in an inactive state sequesters $MAPK^{erk1/2}$ in the cytoplasm. Activation of the $MAPK^{erk1/2}$ module results in MKK^{mek1} phosphorylation and activation of $MAPK^{erk1/2}$. The activation of $MAPK^{erk1/2}$ results in its dissociation from MKK^{mek1}. Activated $MAPK^{erk1/2}$ is rapidly translocated to the nucleus where it is functionally sequestered and can regulate the activity of nuclear proteins including transcription factors. The inactivation of $MAPK^{erk1/2}$ requires its dephosphorylation by specific phosphatases (see below). Nuclear $MAPK^{erk1/2}$ when dephosporylated returns to the cytoplasm and reassociates with MKK^{mek1}.

Transfection analysis and biochemical reconstitution in vitro indicates that $MKKK^{raf1}$ can activate both MKK^{mek1} and MKK^{mek2}. Stimulation of cells through receptor tyrosine kinases, nonreceptor tyrosine kinases, and G protein-coupled receptors also leads to activation of both MKK^{mek1} and MKK^{mek2} (225, 366). There are several conditions where MKK^{mek1} and MKK^{mek2} appear to be differentially regulated. When recombinant Ras · GTP is used to bind effectors from cell lysates, a complex of $MKKK^{raf1}$ and MKK^{mek1} or $MKKK^{B\text{-}raf}$ and MKK^{mek1} is found. MKK^{mek2} has not been isolated in these complexes. Also, cells transformed by oncogenic forms of Ras display increased MKK^{mek1} activity compared with MKK^{mek2}, suggesting that Ras and $MKKK^{raf1}$ preferentially signal to $MAPK^{erk1/2}$ via MKK^{mek1} (161, 225). In response to phorbol esters or endothelin-1 treatment of cardiomyocytes, $MKKK^{A\text{-}raf}$ activated MKK^{mek1} (22). $MKKK^{B\text{-}raf}$ activated MKK^{mek1} in PC-12 cells in response to NGF, EGF, and PDGF. Serum stimulation of NIH 3T3 cells stimulates MKK^{mek1} (190, 366), and $MKKK^{A\text{-}raf}$ selectively activates MKK^{mek1} compared with MKK^{mek2} in response to EGF treatment of HeLa cells (225, 366). Thus MKK^{mek1} and MKK^{mek2} appear to be differentially activated by $MKKK^{raf1}$, $MKKK^{A\text{-}raf}$, and $MKK^{B\text{-}raf}$ in different cell types and in response to different extracellular stimuli. Stimuli that preferentially activate MKK^{mek2} are still to be identified.

There are several reports in the literature that demonstrate $MAPK^{erk1/2}$ activation when $MKKK^{raf}$ activation could not be demonstrated. For example, EGF treatment of Swiss 3T3 cells, insulin stimulation of adipocytes, and tumor necrosis factor-α (TNF-α) challenge of macrophages activated MKK^{mek1} and $MAPK^{erk1/2}$ but not $MKKK^{raf1}$ (133, 225, 374, 404). Because in mouse macrophages neither $MKKK^{B\text{-}raf}$ nor $MKKK^{A\text{-}raf}$ was expressed, these results suggest that a MKKK other than $MKKK^{raf}$ was involved in activating the $MAPK^{erk1/2}$ pathway. Reported activators of $MKK^{mek1/2}$ other than $MKKK^{raf}$ subfamily members include $MKKK^{mos}$, $MKKK^{tpl2}$, $MKKK^{mekk1}$, $MKKK^{mekk2}$, and $MKKK^{mekk3}$ (92) (Fig. 10). A COOH-terminal truncation of $MKKK^{tpl2}$ referred to as $MKKK^{cot}$, $MKKK^{mekk1}$, $MKKK^{mekk2}$, and $MKKK^{mekk3}$ is also capable of activating the $MAPK^{jnk}$ signaling pathway (92). This indicates that some MKK^{mek1} activators can feed into the $MAPK^{jnk}$ pathway by activation of MKK^{sek1}. This finding is reminiscent of budding yeast, where specific MKKK like $MKKK^{ste11}$ can function in more than one MAPK module. Both $MKKK^{mos}$ and $MKKK^{tpl2}$ have restricted patterns of expression; $MKKK^{mos}$ is expressed in germ line cells where it is a key regulator of meiosis and has been found expressed in some cervical cancer cell lines (92, 366), and $MKKK^{tpl2}$ is mainly expressed in lymphoid and hemopoietic cells (92). Thus $MKKK^{mos}$ and $MKKK^{tpl2}$ may serve as tissue-specific activators of MKK^{mek1} or MKK^{mek2}. $MKKK^{mekk1,\ 2\ and\ 3}$ are expressed in a wide variety of tissues and have been shown to activate the $MAPK^{erk1/2}$ pathway in transfection assays. Only kinase-inactive $MKKK^{mekk1}$ and not $MKKK^{mekk2/3}$ has been shown to inhibit receptor stimulation of $MAPK^{erk1/2}$ (93). $MKKK^{mekk1}$ has also been shown to bind to Ras · GTP (296), suggesting that it has the necessary regulatory functions to be an MKKK in the $MAPK^{erk1/2}$ module. In addition to Ras association, $MKKK^{mekk1}$ also binds Rac and Cdc42, which may direct its activity toward the $MAPK^{jnk}$ signaling pathway (93). The implication of these findings is that several different MKKK may function in mammaliam $MAPK^{erk1/2}$ signaling modules. The prediction is that upstream inputs involving MKKKK, GTP-binding proteins, or other signals that are differentially regulated by extracellular stimuli could regulate different $MAPK^{erk1/2}$ modules, allowing discrimination and integration of signals. $MKKK^{mekk1}$ encodes a predicted pleckstrin homology domain that may bind phosphatidylinositol 3,4,5-trisphosphate or phosphatidylinositol 4,5-bisphosphate, reaction products of the phosphatidylinositol 3-kinase (PI3K)-catalyzed reaction. A difference, therefore, between $MKKK^{mekk1}$ and $MKKK^{raf1}$ could be the activation of $MKKK^{mekk1}$ by PI3K reaction products.

Reversal of $MAPK^{erk}$ activation involves dephosphorylation of the Thr(P)-Glu-Tyr(P) activation motif by protein kinase phosphatases. Protein kinase phosphatases that can inactivate MAPK include MKP-1 (also known as CL100, 3CH134, Erp, and hVH-1), MKP-2 (hVH-2, Typ-1), MKP-3 (rVH6, Pyst1), MKP-4, PAC1, VHR, B23 (hVH-3), and M3/6 (hVH-5) (55, 251–253, 366). Among these phosphatases, MKP-1, MKP-3, and MKP-4 are more selective in dephosphorylating and inactivating $MAPK^{erk1/2}$ compared with other

MAPK (100, 122, 252, 253). Except M3/6 that is very specific for $MAPK^{p38}$ and $MAPK^{jnk}$, the other phosphatases do not seem to discriminate between different MAPK. MKP-3 and MKP-4 are primarily in the cytoplasm, whereas the other phosphatases are nuclear. This finding indicates a specific control of the MAPK by MKP-3 and MKP-4 compared with the other phosphatases based on their cellular location.

$MAPK^{erk1/2}$ has many substrates with diverse functions in cells, and the question should be asked what the biological outcome is of $MAPK^{erk1/2}$ activation? The unsatisfying answer is that the role of $MAPK^{erk1/2}$ is probably as variable as there are differentiated cell types. In cell culture systems, there is a good correlation between $MAPK^{erk1/2}$ activation and proliferation of cells. Growth factors such as EGF and PDGF that stimulate proliferation of cells also frequently give a strong and persistent stimulation of $MAPK^{erk1/2}$ activity. Interfering with components of the $MAPK^{erk1/2}$ signaling pathway with dominant negative mutants or antisense constructs for $MKKK^{raf1}$ or $MAPK^{erk1}$ shows significant inhibition of cell proliferation (238, 272, 308). Conversely, constitutively activated MKK^{mek1} persistently stimulates $MAPK^{erk1}$ activity, resulting in enhanced cell proliferation (308).

$MAPK^{erk2}$ activation may not always be required for proliferation. Interleukin-4 stimulation of B cells does not significantly activate $MAPK^{erk}$ signaling but still induces proliferation. Conversely, okadaic acid (a phosphatase inhibitor) treatment of B lymphocytes activates $MAPK^{erk2}$ but inhibits rather than augments cellular proliferation (42, 353), indicating that regulation of the phosphorylation status of other proteins by okadaic acid can have growth inhibitory effects even though $MAPK^{erk2}$ is activated. In smooth muscle cells, activation of $MAPK^{erk1/2}$ leads to PGE_2 secretion that inhibits cellular proliferation. However, in smooth muscle cells lacking the inducible form of cyclooxygenase, activation of $MAPK^{erk1/2}$ does not lead to secretion of PGE_2, and the cells proliferate (30). Therefore, the $MAPK^{erk1/2}$ signaling pathway can mediate either proliferation or growth inhibition depending on the repertoire of genes that are regulated in a specific cell type. Consistent with this theme is the ability of constitutively active MKK^{mek1} or $MAPK^{erk2}$ to induce differentiation in some cell types including PC-12 pheochromocytoma (282) and K562 erythroleukemia cells (283). Treatment of PC-12 cells with either EGF or NGF activates the $MAPK^{erk1/2}$ signaling pathway, yet EGF stimulates proliferation while NGF induces growth arrest and differentiation (282). This apparent difference in the outcome between EGF and NGF treatment has been postulated to be related to differences in the duration and magnitude of the $MAPK^{erk1/2}$ activation. Epidermal growth factor-stimulated $MAPK^{erk1/2}$ activity in PC-12 cells is transient, returning to basal or near-basal levels within 1–2 h. In contrast, the NGF-stimulated activity is more sustained. Overexpression of the EGF receptor in PC-12 cells changes the response to EGF from proliferation to differentiation, a response that is correlated with prolonged $MAPK^{erk1/2}$ activation (76). Taken together, these results indicate that the strength and duration of the signals transmitted through $MAPK^{erk1/2}$ will contribute to determine whether a cell proliferates or differentiates in response to a specific stimulus.

$MAPK^{erk1/2}$ may also contribute to cell cycle regulation in some cell types. In Chinese hamster ovary cells, $MAPK^{erk1/2}$ is activated in the G_1 and M phase of the cell cycle (332). $MAPK^{erk1/2}$ is associated with the microtubule-organizing center (MTOC) during M phase of the cell cycle, suggesting an involvement of $MAPK^{erk1/2}$ in controlling MTOC function (347). Furthermore, $MAPK^{erk1/2}$ function in the spindle assembly checkpoint in *Xenopus* egg extracts (239) (see sect. VI*D*). The substrates for $MAPK^{erk1/2}$ in cell cycle control are not defined. Persistent activation of $MAPK^{erk1/2}$ has also been shown to be required to pass the G_1 restriction point in fibroblasts (194). It was shown that $MAPK^{erk1/2}$ activation increases cyclin D1 promoter activity and cyclin D1 protein expression (193). These results demonstrate that $MAPK^{erk1/2}$ can modulate cyclin D1 expression and its associated cyclin-dependent kinase activities for the control of G_1 progression.

Activation of $MAPK^{erk1/2}$ may also provide protection against apoptosis in some cell types. In PC-12 cells, the withdrawal of NGF led to the inhibition of $MAPK^{erk1/2}$ activity and cell death (383). Constitutive activation of the $MAPK^{erk1/2}$ pathway in these cells inhibited apotosis. In L929 cells, blockage of $MAPK^{erk1/2}$ activation prevents the protection against TNF-α-induced apoptosis that is mediated by fibroblast growth factor (FGF)-2 (108). In Jurkat cells, the ERK pathway is activated when Fas is stimulated (117, 368); however, the ERK activation is transient, possibly as a consequence of $MKKK^{raf1}$ degradation in apoptotic cells (368). It is believed that the function of the inactivation of the $MAPK^{erk1/2}$ response pathway in Fas-stimulated cells is to prevent $MAPK^{erk1/2}$-mediated protection against the apoptotic response. Support for the hypothesis that the $MAPK^{erk1/2}$ pathway components are involved in survival signaling comes from $MKKK^{B\text{-}raf}$ knock-out mice. These mice die of vascular defects during mid-gestation and show increased numbers of endothelial precursors cells, enlarged blood vessels and apoptotis of differentiated endothelial cells (376). Thus $MKKK^{B\text{-}raf}$ appears critical as a signaling element in the development of the vascular system. This appears to be due, at least in part, to its ability to protect maturing endothelial cells from apoptosis. In some cases, however, the $MAPK^{erk1/2}$ pathway may be positively involved in cell death as indicated by the observation that inhibition of MKK^{mek1} activity inhibits crocidolite asbestos (a carcinogen)-induced apoptosis (164) and Fas-induced apoptosis (117). How inhibition of MKK^{mek1} activity would suppress apoptosis is unclear from these studies.

4. *Receptor activation of the $MAPK^{erk1/2}$ pathway*

Many different receptor types are able to activate the $MAPK^{erk1/2}$ pathway (Fig. 11). Upon stimulation of recep-

tor tyrosine kinases such as the EGF receptor, PDGF receptor, or insulin receptor, their intrinsic tyrosine kinase domains are activated, leading to tyrosine phosphorylation of specific substrates including themselves (225, 308, 366). Tyrosine phosphorylation of the receptor allows the binding of adapter proteins to the receptor. Adapter proteins within their amino acid sequence have specific motifs that are involved in protein-protein interactions. The adapter protein Shc consists of a phosphotyrosine binding (PTB) domain, Src homology 2 (SH2) domain [which, as PTB, also binds phosphotyrosine residues], and a Src homology 3 (SH3) domain. After activation of the EGF receptor, Shc binds to a specific phosphotyrosine of the receptor via its PTB domain (80, 225). The association of Shc with the EGF receptor permits the tyrosine phosphorylation of Shc by the receptor itself or intracellular tyrosine kinases such as Src (15). This phosphorylation allows the binding of another adapter protein, Grb2, consisting of a SH2 domain and two SH3 domains (80, 301). The association between Shc and Grb2 is mediated by the SH2 domain of Grb2. Grb2 can also bind directly to the EGF receptor through its SH2 domain (15). These associations amplify the amount of Grb2 associated with the receptor. Because the guanine nucleotide exchange factor Sos (son of sevenless) binds constitutively to the Grb2 SH3 domain, the binding of Grb2 with the EGF receptor also recruits Sos (80, 301). The localization of Sos to the EGFR leads to the exchange of Ras·GDP for GTP at the plasma membrane (80). Ras·GTP interacts with $MKKK^{raf1}$ or other MKKK fitting into the $MAPK^{erk1/2}$ module and leads to the activation of the $MAPK^{erk1/2}$ pathway. The activation of the $MAPK^{erk1/2}$ signaling pathway by other receptor tyrosine kinases involves similar recruitment of adapter proteins leading to Ras activation.

A second group of receptors that activate $MAPK^{erk1/2}$ includes specific cytokine receptors, the T-cell receptor, CD28, and the B-cell receptor. Upon ligation of these receptors, there is a rapid and transient increase in tyrosine phosphorylated proteins. Because these receptors lack an intrinsic tyrosine kinase domain, tyrosine phosphorylation of target proteins is accomplished by the activation of Src family tyrosine kinases including Lck, Lyn, Fyn, and others (46, 333). Similar to receptor tyrosine kinases, the receptor itself becomes tyrosine phosphorylated, enabling the recruitment of the adapter proteins Shc and Grb2 to the cytoplasmic surface of the receptor effectively recruiting Sos to the receptor. These events lead to GTP loading of Ras and $MAPK^{erk1/2}$ activation (46).

Several cytokine receptors activate the $MAPK^{erk1/2}$ pathway through the activation of JAK (Janus kinases including JAK1, -2, -3, and Tyk2). In addition to its ability to phosphorylate and activate STAT, JAK1 can phosphorylate Shc on tyrosines leading to activation of the $MAPK^{erk1/2}$ pathway (375). In turn, $MAPK^{erks}$ may phosphorylate and potentiate the activity of the STAT (375). In human multiple myeloma cells, interleukin (IL)-6 triggers cell growth through the $MAPK^{erk1/2}$ pathway in a JAK- and Ras-dependent manner (267). However, JAK-mediated $MAPK^{erk1/2}$ activation can occur independently of Ras activation. For example, interferon-β induces $MKKK^{raf1}$ activation in HeLa cells in a Ras-independent manner, whereas oncostatin M stimulation of $MKKK^{raf1}$ correlated with GTP loading of Ras (321).

G-protein coupled receptors (GPCR) can also engage the $MAPK^{erk1/2}$ pathway. With many G_i-coupled receptors, treatment of cells with pertussis toxin inhibits $MAPK^{erk}$ activation. When G_i-coupled receptors are involved, $MAPK^{erk1/2}$ activation is believed to be primarily mediated by the $\beta\gamma$-subunit complex. One mechanism for $\beta\gamma$-stimulated $MAPK^{erk}$ activity appears to be PI3Kγ dependent (213). This pathway was shown to be specific for PI3Kγ,

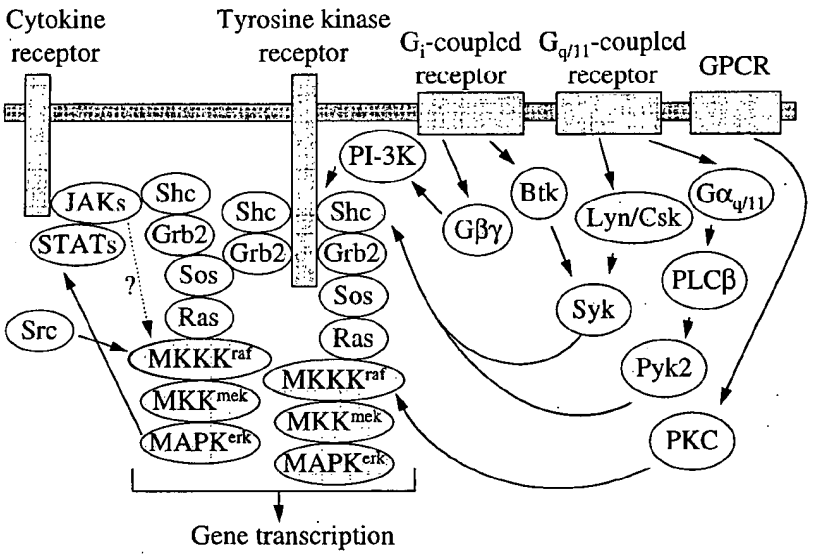


FIG. 11. Receptor–mediated activation of $MAPK^{erk}$ pathway. See text for details. JAK, Janus kinase; STAT, signal transducer and activator of transcription; PLCβ, phospholipase C-β; PI-3K, phosphatidylinositol 3-kinase.

since PI3Kα could not be used as a substitute for PI3Kγ (213). Signaling from PI3Kγ to the MAPK^{erk} pathway required a tyrosine kinase, Shc, Grb2, Sos, Ras, and MKKK^{raf1} (213), indicating that the GPCR that activate the MAPK^{erk} pathway in a PI3Kγ-dependent manner can transactivate receptor tyrosine kinases (Fig. 11). Some GPCR activate the MAPK^{erk} pathway in a pertussis toxin-insensitive manner. This activation mechanism involves the stimulation of $G\alpha_{q/11}$ proteins and the activation of phospholipase C-β. The stimulation of intracellular calcium activates the proline-rich tyrosine kinase (Pyk2) (205). Pyk2 activates Ras through the tyrosine phosphorylation of Shc and/or by recruitment of the Grb2/Sos complex similar to receptor tyrosine kinases (327). In response to endothelin-1, lysophosphatidic acid, or thrombin, the EGF receptor is rapidly tyrosine-phosphorylated, leading to the recruitment of adaptor proteins such as Grb2 (69, 327). This recruitment eventually leads to MAPK$^{erk1/2}$ activation as described above. Inhibition of the EGF receptor function suppressed MAPK$^{erk1/2}$ activation by the GPCR (69). It has also been suggested that the GPCR-induced tyrosine phosphorylation of EGF receptor is mediated by a Src tyrosine kinase (215).

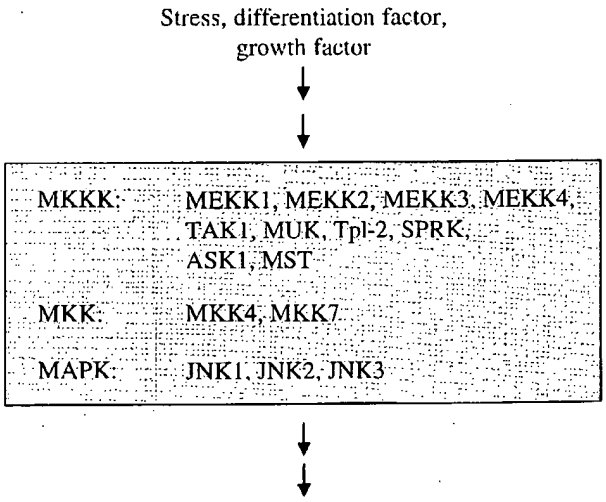
In lymphoid cells, targeted deletion of Lyn or Csk tyrosine kinases blocks the stimulation of MAPK$^{erk1/2}$ by G_q- but not G_i-coupled receptors (350, 351). In cells deficient for Btk tyrosine kinase, G_i-coupled receptors failed to activate ERK, whereas G_q-coupled receptor-mediated stimulation was unaffected (350). Cells lacking Syk were deficient in both G_q- and G_i-mediated MAPK^{erk} stimulation (350, 351). Syk appears to integrate the signals stimulated by different GPCR, leading to the activation of the MAPK$^{erk1/2}$ pathway (Fig. 11). In addition, there is evidence that some GPCR activate MAPK$^{erk1/2}$ in a PKC-dependent, Ras-independent manner (366).

Finally, the MAPK^{erk} pathway can be activated by engagement and clustering of integrins. Integrins are a family of transmembrane receptors that bind to proteins of the extracellular matrix, such as fibronectin, collagen, and vitronectin. Binding and clustering of integrins leads to the formation of focal adhesion structures, in which integrins connect to actin stress fibers. Integrin ligation induces the activation of a variety of signaling events, including the activation of the MAPK$^{erk1/2}$ pathway (50, 242, 249, 407). The activation of the MAPK$^{erk1/2}$ after integrin ligation is mediated in part by Rho (290). Cell adhesion is also important for survival of adherent cells. The regulation of the MAPK$^{erk1/2}$ pathway by integrins may contribute to the survival response observed with integrin-mediated adherence.

B. MAPK^{jnk} Pathway

A new MAPK was identified biochemically in 1991 (281) that was distinguished from the MAPK^{erks} by two characteristics: *1*) it was activated by cell stress such as ultraviolet irradiation, and *2*) it phosphorylated c-Jun at the NH_2-terminal activating sites rather than the COOH-terminal inhibitory sites phosphorylated by the MAPK^{erk2}. When the gene encoding this kinase was cloned by two groups, the human homolog was named c-Jun NH_2-terminal kinase (MAPK^{jnk}) (73, 188), and the rat homolog was named stress-activated protein kinase (SAPK) (188). Two more genes encoding MAPK^{jnk} family members have also been cloned (123, 169, 188). These members of the MAPK^{jnk} group of MAPK, along with their MKKK and MKK, form the MAPK^{jnk} module (Fig. 12).

Differential splicing and exon usage yield a total of 10 different MAPK^{jnk} isoforms from the 3 genes (123). Each kinase is expressed as a short form and a long form, and MAPK^{jnk1} and MAPK^{jnk2} have an alternative sequence that appears in the kinase domain in some transcripts. A definitive analysis of the expression patterns of these 10 different isoforms in vivo has not been done. MAPK^{jnk1} and MAPK^{jnk2} are expressed ubiquitously, whereas the expression of MAPK^{jnk3} appears to be limited to the brain (390). The alternative forms of the MAPK^{jnks} differ in their ability to bind, and presumably activate, different transcription factors (123). There is also evidence that the different forms of the enzyme can be differently regulated. In mouse macrophages stimulated with TNF-α, a 46-kDa MAPK^{jnk} is activated, whereas a 54-kDa isoform is not (47). Because of extensive sequence homology between



Components of the MAPK^{jnk} pathways.

FIG. 12. MKKK, MKK, and MAPK that can be components of MAPK^{jnk} pathway.

these paralogues, biochemical identification using antibodies is very difficult, if at all possible. Whether these measurable differences reflect a real effect on the determination of cell fate remains to be determined.

$MAPK^{jnk}$ was first described as a SAPK, and it is the response to stress that has been most widely studied. In spite of the volume of research in this area, little is known about the signal transduction pathways leading from cell stress, such as ultraviolet irradiation, to the activation of the $MAPK^{jnk}$ cascade. Cell stress can come in several forms, including heat shock, direct damage of DNA (γ-irradiation, cytosine arabinoside), generation of reactive oxygen species (H_2O_2), and conditions of hyperosmolarity. Until the exact sensors of cell stress are identified and characterized, the pathways that lead from stress to activation of the cascade will remain elusive. Cellular redox state, tyrosine kinases, and phosphatases are potentially involved in some stress responses, but the mechanisms for regulation have not yet been defined.

The $MAPK^{jnk}$ response to extracellular ligands is far better characterized. $MAPK^{jnks}$ can be activated through different receptor types, and the signal transduction pathways that can converge on the $MAPK^{jnk}$ cascade vary widely. $MAPK^{jnks}$ have been shown to be activated through cell surface receptors from a variety of families, including the TNF receptor family, GPCR, tyrosine kinase receptors, and cytokine receptors. The growing list of signaling proteins that are capable of activating the $MAPK^{jnk}$ pathway has been recently reviewed (92).

$MAPK^{jnks}$ are activated by phosphorylation on threonine and tyrosine of the Thr-X-Tyr activation motif by one of two cloned dual specificity kinases, MKK^{mkk4} (300) and MKK^{mkk7} (340). These kinases are in turn activated by an MKKK, of which several examples have been identified (Fig. 12); $MKKK^{mekk1-4}$ (20, 111, 191), $MKKK^{ask1}$ (357), $MKKK^{tak1}$ (387), $MKKK^{mst}$ (138), $MKKK^{sprk}$ (286), $MKKK^{muk}$ (137), and $MKKK^{tpl2}$ (298) have been shown to phosphorylate MKK^{mkk4} and activate its kinase activity and thus are designated as MKKK for the $MAPK^{jnk}$ pathway.

Other kinases are capable of activating $MAPK^{jnks}$ when they are overexpressed in cells but have not been demonstrated to act as MKKK. In yeast, Ste20 is often placed directly upstream of the $MKKK^{mekk1}$ homolog $MKKK^{ste11}$ (note again that this has yet to be proven genetically and biochemically). Therefore, Ste20 homologs, such as p21-activated kinase, germinal center kinase (GCK), Nck-interacting kinase (NIK), hematopoietic progenitor kinase 1 (HPK1), and GCK-like kinase (GLK) may regulate $MAPK^{jnk}$ by phosphorylation of specific MKKK. However, despite a great deal of interest in these kinases and the $MAPK^{jnk}$ pathway, there has never been a direct demonstration of the activation of a MKKK by a Ste20 homolog. Although these kinases may activate $MAPK^{jnk}$ when overexpressed in cells, their regulation of different MKKK has not been demonstrated biochemically or genetically. This is the case for NIK and HPK1 that, although capable of binding $MKKK^{mekk1}$ and $MKKK^{sprk}$, respectively (175, 326), have not been shown to directly activate $MKKK^{mekk1}$ and $MKKK^{sprk}$.

Other signaling proteins that act as upstream activators of the $MAPK^{jnk}$ module include the low-molecular-weight GTP-binding proteins of the Rho family. These proteins, in particular Rac and Cdc42, have been demonstrated to activate $MAPK^{jnk}$ when expressed as constitutively active forms. The competitively inhibitory mutant forms of Rac and Cdc42 can block $MAPK^{jnk}$ activation induced by EGF or TNF-α (62). They may do so by binding to a MKKK (93, 111) or to one of the Ste20 homologs discussed above (11).

$MAPK^{jnk}$ activities can be downregulated by dual-specificity protein phosphatases, including M3/6 (also known as hVH-5) (253) and MKP-1 (also known as CL100, 3CH134, Erp, and hVH-1) (100, 140). These phosphatases display selectivity toward $MAPK^{jnk}$ family members. MKP-1 has been shown to be induced by MAPK pathways. However, there are conflicting results as to which MAPK pathway is responsible for this induction. In U 937 cells, $MAPK^{erk2}$, but not $MAPK^{p38\alpha}$ or $MAPK^{jnk1}$, mediates the induction of MKP-1 expression (100). In contrast, in NIH 3T3 fibroblasts, the $MAPK^{jnk}$ pathway, but not the $MAPK^{erk}$ pathway, induces MKP-1 induction (25). It is possible that this difference is because of the different cell lines used, but further work is required to clarify this issue. The upstream regulation of M3/6 has not been demonstrated, so it is not known whether the phosphatase is a selective point of $MAPK^{jnk}$ regulation or a constitutively active switch that turns off $MAPK^{jnk}$ activity when the upstream activators are silenced.

The expression of another protein, $MAPK^{jnk}$ interacting protein-1 (JIP-1) causes a decrease in the $MAPK^{jnk}$ activation of transcription factors (75), suggesting that JIP-1 acts as an inhibitor of $MAPK^{jnk}$. Several other biochemical and biological functions of $MAPK^{jnk}$ are inhibited by a fragment of JIP-1 (the $MAPK^{jnk}$ binding domain, or JBD), including activation of c-Jun and Elk-1, transformation of cells by break point cluster region-Abelson proto-oncogene (Bcr-Abl), and apoptosis induced by NGF withdrawal. However, it is not obvious that the inhibitory functions of the fragment are physiological functions of the full-length protein or merely a consequence of a loss of a functional domain of the protein. The full-length JIP-1 acts as a $MAPK^{jnk}$ substrate and may have downstream activities of its own, which are blocked by overexpression of the $MAPK^{jnk}$ binding domain. Recently, the rat homolog of JIP-1 (IB-1) has been cloned (27). Compared with the mouse JIP-1 sequence, IB-1 encodes a 47-amino acid insertion in a phosphotyrosine interaction domain. Polymerase chain reaction analysis of mouse and rat tissues has only revealed the sequences corresponding to IB-1 (that is the sequence with 47-amino acid insertion) (G. Waeber, per-

sonal communication), raising the possibility that JIP-1 is a truncated version of the natural protein.

The $MAPK^{jnk}$ substrates are, to date, exclusively transcription factors, in contrast to the $MAPK^{erk}$ family that appears to have substrates outside the nucleus. The substrates that have been identified for $MAPK^{jnks}$ include c-Jun, ATF-2 (124), Elk-1 (409), p53 (145, 237), DPC4 (3), and NFAT4 (54). Phosphorylation of c-Jun at serine-63 and serine-73 by $MAPK^{jnk}$ results in an increase in the formation of Jun/Jun homodimers and Jun/ATF2 heterodimers. c-Jun phosphorylated by the $MAPK^{jnks}$ is also more resistant to ubiquitin-dependent degradation (256). Thus the $MAPK^{jnks}$ have the abilility to activate transcription factors as well as stabilizing them, resulting in efficient activation of the target genes controlled by the $MAPK^{jnk}$-regulated transcription factors. Phosporylation of ATF-2 by $MAPK^{jnk}$ also leads to an increase in transcriptional activity. The transcription factor Elk-1 represents a point of convergence for the $MAPK^{erk}$ and $MAPK^{jnk}$ pathways (409). Phosphorylation of NFAT4 by $MAPK^{jnk}$ inhibits its function by preventing its translocation to the nucleus (54). Mutation of the serines that $MAPK^{jnk}$ phosphorylates leads to a NFAT4 mutant that is constitutively active and located in the nucleus (54). The inhibitory function of $MAPK^{jnk}$ on NFAT4 can be opposed by the phosphatase calcineurin (54). DPC4 is a human mothers against decapentaplegic (Mad)-related transcriptional factor regulated by transforming growth factor-β (TGF-β) in a $MAPK^{jnk}$ pathway-dependent manner (3). Overexpression of DPC4 leads to apoptosis, and this response can be potentiated by mad3, another transcription factor that associates with and is phosphorylated by the TGF-β receptor (401). This suggests that mad3 links the TGF-β receptor to DPC4 activation and possibly apoptosis. Finally, the transcription factor p53 is phosphorylated on serine-34 by all three isoforms of $MAPK^{jnk}$ (145). However, the functional significance of this phosphorylation is unclear.

The mechanism by which $MAPK^{jnk}$ recognizes these substrates involves a bipartite sequence. As with the other MAPK, $MAPK^{jnk}$ phosphorylates substrates at a Ser/Thr-X-Pro motif. However, this sequence is not sufficient for $MAPK^{jnk}$ to phosphorylate a protein, because JunB contains such a site and is not efficiently phosphorylated by $MAPK^{jnk}$ (123). An additional docking site that is present on c-Jun is necessary for phosphorylation of that protein. The recruitment of $MAPK^{jnk}$ to this docking site effectively increases the local concentration of the kinase and directs activity to the proper NH_2-terminal phosphorylation motif on c-Jun. Because c-Jun has the ability to heterodimerize with other AP-1 components, the binding of $MAPK^{jnk}$ to this docking site allows the kinase to come into contact with transcription factors that lack a docking site of their own (169). It has been suggested that the differences between the activities of $MAPK^{jnk1}$ and $MAPK^{jnk2}$ are explained by their differing affinities for c-Jun. $MAPK^{jnk2}$ shows a much higher affinity to c-Jun because of a specificity-determining region that $MAPK^{jnk1}$ lacks (169). $MAPK^{jnk}$ also binds to ATF-2 at a site just NH_2 terminal to the phosphorylation motif, and thus it interacts with this transcription factor in a bipartite manner as well (124).

$MAPK^{jnk}$ activity has been implicated in the response to cell stress, specifically apoptosis. Although it has never been demonstrated that a $MAPK^{jnk}$ is sufficient for apoptosis in any system, it seems necessary for this process to occur in at least some cases (77). Inhibition of $MAPK^{jnk}$ signaling by introduction of dominant inhibitory mutants of $MAPK^{jnk1}$, its main downstream target c-Jun, or its major upstream activator MKK^{mkk4}, show that $MAPK^{jnk}$ is necessary for apoptosis in the response to growth factor withdrawal (125, 383), stress (51), DNA damage (170), and ligation of Fas on the cell surface (117). Depending on the cell type, Fas-induced apoptosis may or may not necessitate the activation of the $MAPK^{jnk}$ pathway (391). Whether the $MAPK^{jnk}$ pathway is required for Fas-induced apoptosis could depend on the cell's sensitivity to the death response and/or the presence of Daax, a Fas-binding protein that activates $MAPK^{jnk}$ (391).

When the $MAPK^{jnk}$ pathway is required for apoptosis, it is expected that c-Jun-dependent transcription leads to the de novo synthesis of pro-apoptotic proteins. Characterization of the pro-apoptotic proteins that are induced by MAPK pathways is clearly an important step in elucidating the role of these signaling events in apoptosis. Expression of Fas ligand (FasL) is one pathway of transcription-dependent apoptosis. In response to DNA damage, FasL is expressed at the surface of T cells where it activates Fas and consequently induces apoptosis. Deoxyribonucleic acid damage-induced FasL expression necessitates the activation of the AP1 transcription factors in a $MAPK^{jnk}$-dependent manner (170). Expression of FasL establishes thus a direct link between activation of the $MAPK^{jnk}$ pathway and apoptosis. $MKKK^{mekk1}$ appears to be the MKKK in the $MAPK^{jnk}$ module that is required for FasL expression (97, 170). Interestingly, $MKKK^{mekk1}$ has also the potential to activate the NFκB transcription factor (139, 196). Because NFκB is also required for DNA damage-induced FasL expression (the FasL promoter contains AP-1 and NFκB binding sites) (170), it is possible that $MKKK^{mekk1}$ integrates the stress signals that lead to activation of both AP1 and NFκB.

Yang et al. (390) demonstrated a specific role for $MAPK^{jnk3}$ in excitotoxicity. In $MAPK^{jnk3}$-deficient mice, administration of an epileptogenic dose of kainic acid did not cause seizures that were as severe as those in similarly treated wild-type mice. The onset of seizures coincided with cell death in an area of the hippocampus. No cell death occurred in this area in the $MAPK^{jnk3}$ knockout mice. This is probably the best evidence for the role of a specific $MAPK^{jnk}$ in an apoptotic response. The function

of $MAPK^{jnk3}$ in this excitotoxicity is undefined. Despite these observations, other investigators have suggested that $MAPK^{jnk}$ are not involved in apoptotic responses (160, 211). The discordance is mostly related to Fas- and TNF-α receptor-mediated apoptosis, where the receptors may regulate caspases by a pathway different from that of other cellular stresses.

$MKKK^{mekk1}$, an upstream regulator of the $MAPK^{jnk}$ pathway, is able to regulate the apoptotic response possibly in a $MAPK^{jnk}$-independent manner (192, 367). In its full-length 196-kDa form, $MKKK^{mekk1}$ does not seem to promote apoptosis (41, 367). In fact, activation of full-length $MKKK^{mekk1}$ may induce survival responses, including the activation of the $MAPK^{erk}$ and NFκB pathways (139, 191, 196). Both $MAPK^{erk}$ and NFκB pathways have been shown to have survival responses in different cell types (see sect. v*A3* and Refs. 229, 345, 352). $MKKK^{mekk1}$ is a caspase-3 substrate, and when cleaved into a 91-kDa kinase fragment during apoptosis, it becomes a strong apoptotic inducer (41, 192, 367, 369). The cleavage of $MKKK^{mekk1}$ could thus switch the function of $MKKK^{mekk1}$ from a protective response to a cell death-promoting response.

Although $MAPK^{jnk}$ in several cell lines appears to mediate a stress response that is often associated with subsequent cell death, in some cases it may act to promote survival or growth. BAF3 pre-B cells undergo apoptosis when deprived of IL-3. Readdition of IL-3 stimulates a $MAPK^{jnk}$ response in these cells, and inhibition of $MAPK^{jnk}$ activity by expression of a $MAPK^{jnk}$ specific phosphatase inhibits IL-3 induced proliferation, while having no effect on apoptosis induced by IL-3 withdrawal (318). In T98G glioblastoma cells, the $MAPK^{jnk}$ pathway seems to regulate DNA repair, and its inhibition sensitizes the tumor cells to cisplatin-induced death (279).

In both T and B cells, presentation of an antigen to the antigen receptors [the T-cell receptor (TCR) and the B-cell receptor, or surface IgM] alone causes growth arrest and/or apoptosis. However, presentation of antigen by an antigen-presenting cell allows the coactivation of the antigen receptor and a second receptor, CD28 in the T cell and CD40 in the B cell, that rescues the cell and allows it to proliferate. $MAPK^{jnk}$ has been demonstrated to play a role in this signal integration. Ligation of the TCR or CD28 alone is unable to activate $MAPK^{jnk}$ or induce the synthesis of IL-2. Ligation of both receptors simultaneously activates $MAPK^{jnk}$ and IL-2 production (325). Blocking the $MAPK^{jnk}$ pathway with dominant negative $MKKK^{mekk1}$ blocks IL-2 production in Jurkat cells in response to phorbol ester and calcium ionophore, which can substitute for CD28 ligation in these cells (96). Finally, in lymphocytes that are lacking MKK^{mkk4}, CD28 and CD3 ligation (a similar treatment to TCR ligation) are not as capable of inducing IL-2 production (265). However, although these cells are completely lacking a $MAPK^{jnk}$ response to phorbol ester and calcium ionophore, they are still capable of proliferating and making IL-2, albeit at a lower rate. This suggests that some $MAPK^{jnk}$-independent signal is operating in the proliferative response to these signals. What must be remembered is that none of the MAPK pathways including $MAPK^{jnk}$ is acting alone in these responses; rather, the MAPK pathways are integrated with many other metabolic changes in the cell. The sum of these responses and their integration ultimately determines cell fate.

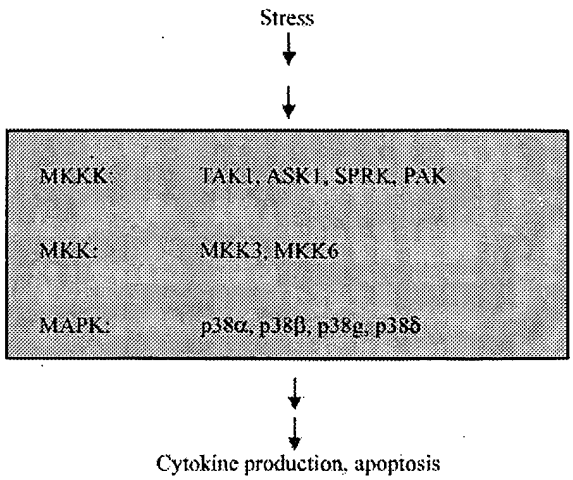


FIG. 13. MKKK, MKK, and MAPK that can be components of $MAPK^{p38}$ pathway.

C. $MAPK^{p38}$ Pathway

The mammalian $MAPK^{p38}$ family consists of at least four different homologous proteins, $MAPK^{p38\alpha}$, $MAPK^{p38\beta}$, $MAPK^{p38\gamma}$, and $MAPK^{p38\delta}$ (163, 323, 366). These $MAPK^{p38}$ have the greatest homology to the yeast $MAPK^{hog1}$, which is activated by hyperosmotic shock (366). Similarly to $MAPK^{hog1}$, different $MAPK^{p38}$ are activated by cellular stress (ultraviolet irradiation, osmotic shock, heat shock, lipopolysaccharide, protein synthesis inhibitors), certain cytokines (IL-1, TNF-α), and GPCR (366). The activation of $MAPK^{p38}$ through either cellular stress or ligation of cell surface receptors involves the activation of specific protein kinases in an ordered activation module (MKKK/MKK/MAPK) (Fig. 13).

Similar to $MAPK^{erk}$ and other MAPK families, $MAPK^{p38}$ is activated by dual phosphorylation of Thr and Tyr in the Thr-Gly-Tyr activation motif (284, 366). Upon stimulation of $MAPK^{p38}$ activity, several specific substrates are phosphorylated. $MAPK^{p38}$ can phosphorylate and activate the MAPK-activated protein (MAPKAP) ki-

nase 2 and 3 which phosphorylate small heat-shock proteins such as 27-kDa heat-shock protein (295). Other substrates for $MAPK^{p38}$ have been identified as transcription factors. ATF2 is phosphorylated by $MAPK^{p38}$ at Thr-69 and Thr-71 within its NH_2-terminal activation domain, resulting in increased transcriptional activity (42, 284). Elk1, an effective $MAPK^{erk}$ substrate, also can be phosphorylated by $MAPK^{p38}$ on several sites in its COOH-terminal activation domain (285). Chop (also known as GADD153) is a member of the C/EBP family of transcription factors that is phosphorylated by $MAPK^{p38}$ on serine residues 78 and 81 (355). Phosphorylation of these residues enhances the ability of Chop to function as a transcriptional activator. Finally, the transcription factor Max binds to a COOH-terminal truncated isoform of $MAPK^{p38\alpha}$, leading to phosphorylation of Max (397). Max heterodimerizes with c-Myc, a $MAPK^{erk}$ substrate, raising the possibility that c-Myc/Max heterodimers represent a point of integration between the $MAPK^{erk}$ and $MAPK^{p38}$ signaling pathways.

Because $MAPK^{p38}$ can phosphorylate many different substrates, it is reasonable to suggest that $MAPK^{p38}$ affects many different biological functions. To investigate biological functions of $MAPK^{p38}$, a kinase inhibitor of $MAPK^{p38}$ [pyridinyl-imidazole compound SmithKline Beecham (SB)-203580] is often used. It was found that administration of SB-203580 blocks production of cytokines (IL-1 and TNF-α) in stimulated monocytes (197). Furthermore, blockage of $MAPK^{p38}$ activity inhibits IL-2 production in T cells (361). This suggests that $MAPK^{p38}$ is important for the production of cytokines in hematopoietic cells. In addition, inhibition of $MAPK^{p38}$ by SB-203580 prevents IL-2- and IL-7-driven proliferation, indicating a role for $MAPK^{p38}$ in cytokine-stimulated cellular proliferation (64). $MAPK^{p38}$ is also implicated in apoptosis. Several cellular stresses such as osmotic shock and ultraviolet irradiation that cause apoptosis activate $MAPK^{p38}$ kinase activity (366). In addition, death receptors (Fas and TNF receptors) upon ligation cause activation of $MAPK^{p38}$ (34). Blockage of $MAPK^{p38}$ activation by pyridinyl-imidazole compounds impairs Fas-induced apoptosis in T cells. Overexpression of $MKKK^{ask1}$, which activates $MAPK^{p38}$ and $MAPK^{jnk}$ activity, induces apoptosis, and TNF-induced apoptosis is blocked by kinase dead $MKKK^{ask1}$ (149). In addition, treatment of cells with sodium salicylate induces apoptosis and activates $MAPK^{p38}$ activity. This induction of apoptosis was blocked by SB-203580 (306). Just as the case with $MAPK^{jnk}$, there are cells where $MAPK^{p38}$ is activated without apoptosis (329). Thus the involvement of $MAPK^{p38}$ in apoptosis may be specific to the type of induction of apoptosis and the cell type used. It is likely that the involvement of $MAPK^{p38}$ in survival versus death responses is integrated into the sum of metabolic changes that determine if the activation of caspases will irreversibly commit a cell to apoptosis.

The function of $MAPK^{p38}$ in differentiated cells is poorly defined. $MAPK^{p38}$ is activated by thrombin in platelets (183), suggesting that $MAPK^{p38}$ plays a role in platelet activation, but no definitive studies have been reported. The $MAPK^{p38}$ pathway may be involved in the cardiac hypertrophic growth program, since expression of an active form of MKK^{mkk6}, which activates $MAPK^{p38}$, leads to augmentation of cell size, induction of the genes encoding A- and B-natriuretic peptides, and increase of α-skeletal actin expression. The active form of MKK^{mkk6} also elicited sarcomeric organization (396).

Regulation of MAPK activation can occur through the activation of small G proteins of the Rho family. In the $MAPK^{jnk}$ pathway, Cdc42 and Rac1 mediate $MAPK^{jnk}$ activation (366). Coexpression of constitutively active forms of Cdc42 and Rac with $MAPK^{p38}$ leads to increased activity of $MAPK^{p38}$. Expression of dominant negative Cdc42 and Rac inhibits the ability of IL-1 to increase $MAPK^{p38}$ activity. These findings suggest that Cdc42 and Rac mediated $MAPK^{p38}$ activation (11, 366, 398). Because Cdc42 and Rac do not directly activate $MAPK^{p38}$ activity, they must activate other signaling molecules leading to $MAPK^{p38}$ activation. These small G proteins have been shown to activate a family of serine and threonine kinases called p21-activated kinase (Pak) (176, 221). Expression of constitutively active Pak stimulates $MAPK^{p38}$ activity, and a dominant negative Pak suppresses IL-1-induced $MAPK^{p38}$ activity (11, 398). Coexpression of Cdc42 or Rac with dominant negative Pak suppresses Cdc42/Rac-mediated $MAPK^{p38}$ activation (11, 398). Thus Cdc42 and Rac regulate the $MAPK^{p38}$ signaling pathway in response to at least some cellular stimuli such as IL-1, and this apparently occurs through activation of Pak. Pak also associates with the adapter protein Nck through its SH3 domain, which localizes Pak to membranes causing its activation (214). Nck could thus function to link activated surface receptors to the Cdc42/Pak signaling pathway and consequently $MAPK^{p38}$ activation. However, there is no evidence indicating that membrane localization of Pak leads to $MAPK^{p38}$ activation. Moreover, no proteins downstream of Pak that could link Pak to the activation of $MAPK^{p38}$ have been characterized. There are, however, additional MKKK candidates for $MAPK^{p38}$ activation. $MKKK^{tak1}$ is activated by Tab1 (Tak1-binding protein) mediating TGF-β signal transduction, and the expression of $MKKK^{tak1}$ in cells leads to $MAPK^{p38}$ activation (354). Another candidate is $MKKK^{ask1}$, which upon expression in cells activates $MAPK^{p38}$ activity. $MKKK^{ask1}$ is activated in response to TNF-α treatment of cells that also leads to $MAPK^{p38}$ and $MAPK^{jnk}$ activation (149). Finally, $MKKK^{sprk}$ may also activate $MAPK^{p38}$ activity upon expression in cells (335) (Fig. 13). The connection between these MKKK to Cdc42/Rac-mediated activation of $MAPK^{p38}$ is unknown, but it appears there will be multiple protein kinases leading to $MAPK^{p38}$ activation similar to the $MAPK^{erk}$ and $MAPK^{jnk}$ signaling pathways.

The protein kinases responsible for phosphorylation

and activation of $MAPK^{p38}$ are $MAPKK^{mkk3}$ and $MAPKK^{mkk6}$ (247, 366) (Fig. 13). Both of these MKK are activated by $MKKK^{tak1}$, $MKKK^{ask1}$, and $MKKK^{sprk}$. $MKKK^{sprk}$ associates with MKK^{mkk6} and phosphorylates amino acid residues on MKK^{mkk3} required for its activation (335).

Hints in the literature suggest that some ligands can activate one MAPK pathway and at the same time inhibit another MAPK pathway. For example, glia maturation factor activates the $MAPK^{p38}$ pathway and at the same time inhibits the $MAPK^{erk}$ pathway. Glia maturation factor is a 17-kDa brain protein that, when phosphorylated by the cAMP-regulated PKA, specifically enhances the activity of $MAPK^{p38}$ (209). On the other hand, PKA-phosphorylated glia maturation factor is a strong inhibitor of $MAPK^{erk1}$ and $MAPK^{erk2}$ (394). This indicates that a PKA-dependent pathway is able to differentially regulate the activity of MAPK pathways.

Just as for the $MAPK^{erk}$ pathway, GPCR in addition to the thrombin receptor can induce the activity of $MAPK^{p38}$. In HEK 293 cells, $MAPK^{p38}$ can be activated through the G_q/G_{11}-coupled m1 muscarinic receptor, through the G_i-coupled m2 muscarinic receptor, and through the G_s-coupled β-adrenergic receptor. Overexpression of $G\beta\gamma$ or $G_{11}\alpha$, but not $G_s\alpha$ or $G_i\alpha$, can stimulate $MAPK^{p38}$ (389). Thus, depending on the GPCR, $MAPK^{p38}$ activation can be mediated by the $\beta\gamma$-subunit complex or the α-subunit of heterotrimeric G proteins.

$MAPK^{p38}$ family members are negatively regulated by protein kinase phosphatases. These phosphatases dephosphorylate the Thr-Gly-Tyr motif in $MAPK^{p38}$, thereby inhibiting its kinase activity. There are some protein kinase phosphatases that inhibit the activity of all MAPK, whereas others are specific (366). The protein kinase phosphatase M3/6 inhibits the activity of p38 and $MAPK^{jnk}$ family members but is a poor inhibitor of the $MAPK^{erk}$ family members (253) (see sect. v*A3*).

D. MKK5/$MAPK^{erk}$ Pathway

The least known mammalian MAPK pathway employs MKK^{mkk5} and $MAPK^{erk5}$. The MKKK in this MAPK module has not been yet identified (87). $MAPK^{erk5}$ can be activated by oxidative stress and hyperosmolarity (1), but also by nonstress stimuli such as serum (172). $MAPK^{erk5}$ can bind MKK^{mkk5} in vitro (405) and, in contrast to $MAPK^{erk2}$, $MAPK^{p38\alpha}$, and $MAPK^{jnk1}$, is activated in vivo by expression of an activated mutant of MKK^{mkk5} (172). This indicates that MKK^{mkk5} is the upstream regulatory kinase of $MAPK^{erk5}$. The activation of $MAPK^{erk5}$ induced its translocation from the cytosol to the nucleus. In response to serum, the activation of MKK^{mkk5} and $MAPK^{erk5}$ leads to the activation of the transcription factor MEF2C, which induces the expression of c-Jun (172). MEF2C can also be regulated by the $MAPK^{p38}$ pathway in macrophages induced with gram-negative bacterial lipopolysaccharide (127). This shows again that different mammalian MAPK pathways can have similar downstream targets, as seen in yeast. However, the p38 and the $MKK^{mkk5}/MAPK^{erk5}$ pathway activates MEF2C through the use of different phosphorylation sites (172). This suggests that MEF2C could integrate, in a noncompetitive fashion, signals originating from different MAPK pathways. Additional work is required to define the other components and signaling proteins that regulate the $MAPK^{erk5}$ module.

VI. MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS IN OTHER ORGANISMS

Components of MAPK pathways are rapidly being characterized in a number of nonmammalian experimental systems, including *X. laevis*, *Drosophila melanogaster*, *Caenorhabditis elegans*, and plant species. The role played by MAPK pathways in these organisms is still fragmentory, but because they represent powerful experimental systems, they will undoubtely provide many interesting insights on the function and role of MAPK pathways in important physiological processes (i.e., development). To illustrate this point, we very briefly discuss MAPK modules and their regulation in nonmammalian and nonyeast organisms.

A. *Dictyostelium discoideum*

In *Dictyostelium*, three independent MAPK pathways regulate growth and multicellular development. The *Dictyostelium* MEK (MKK^{Ddmek1}) is required for chemotaxis toward cAMP during aggregation (216). MKK^{Ddmek1} is required at the time of cAMP stimulation for the activation of guanylyl cyclase and the production of cGMP, the second messenger for chemotaxis in these cells. *Dictyostelium* $MAPK^{erk1}$ is required for vegetative growth and probably plays a role during multicellular development (110). The *Dictyostelium* $MAPK^{erk2}$ is activated by extracellular cAMP and is required for receptor activation of adenylyl cyclase during aggregation, prespore-specific gene expression, and morphogenesis in a partially GPCR-dependent manner (218, 307). The chemoattractant folic acid, which allows the amoebae form of *Dictyostelium* to find bacteria in the wild, activates $MAPK^{erk2}$. This response requires the *Dictyostelium* Gα protein (Gα4) (219). In *Dictyostelium*, there are thus two independent pathways to $MAPK^{erk2}$, one that is mediated by folic acid and is totally dependent on G protein-dependent pathways and one that is mediated by cAMP and is partially G protein independent.

B. *Caenorhabditis elegans*

The ras homolog of *C. elegans* (Let-60) is a key player in the signal transduction pathway that controls the

choice between vulval and epidermal differentiation in response to extracellular signals. The pathway downstream of Let-60 is composed of a MAPK module employing the $MKKK^{raf1}$ homolog $MKKK^{lin45}$ (129), the MKK^{mek} homolog $cMKK^{mek2}$ (181, 381), and the $MAPK^{erk}$ homolog $MAPK^{sur1}$ (380). $MAPK^{sur1}$ acts upstream of the transcription factor Lin-1 (17, 380). Worms deficient in $MAPK^{sur1}$ can be rescued by rat $MAPK^{erk2}$, demonstrating the conservation of a Ras-mediated pathway between worms and mammals. Another $MKKK^{raf1}$ homolog, $MKKK^{ksr1}$, has been isolated by screening suppressors of the multi-vulva phenotype caused by an activated Let-60 Ras allele (328). It is, however, unclear whether $MKKK^{ksr1}$ lies downstream of Let-60 Ras or if it is part of a MAPK pathway that functions in parallel to the $MKK^{lin45}/MAPK^{sur1}$ pathway.

C. *Drosophila melanogaster*

The gene product encoded by the rolled locus is a homolog of the mammalian $MAPK^{erk}$ proteins. This kinase, $dMAPK^{rolled}$, is required for the sevenless receptor tyrosine kinase expressed in the R7 precursor cells to transduce the signal that leads to the development of the R7 photoreceptor cell (19). The *Drosophila* homolog of $MKKK^{raf1}$, $dMKKK^{raf1}$, is required for early larval development (263), indicating that the *Drosphila* $MAPK^{erk}$ pathway is involved in this process. The *Drosophila* $MAPK^{erk}$ pathway seems to be involved in several developmental pathways in the fruit fly.

Dorsal closure, a morphogenetic process occurring during *Drosophila* embryogenesis, is regulated by the gene products of hemipterus ($dMKK^{hep}$) and basket ($dMAPK^{bsk}$), the homologs of MKK^{mkk7} and $MAPK^{jnk1}$, respectively. Embryos lacking these kinases exhibit a dorsal closure phenotype (292). Similarly, embryos lacking the *Drosophila* Jun homolog (Djun) also have a defect in dorsal closure (143). This suggests that Jun is a target of $dMKK^{hep}/dMAPK^{bsk}$ signaling. However, the role of $dMAPK^{bsk}$ in Djun regulation has been disputed (143). $dMAPK^{bsk}$ may also be implicated in an immune response toward bacterial infection in *Drosophila* because it is activated by lipopolysaccharide, a component of the bacterial cell wall (317).

$MAPK^{p38}$ *Drosophila* homologs ($dMAPK^{p38a}$ and $dMAPK^{p38b}$) have recently been cloned (126, 130). Expectedly, the $dMAPK^{p38}$ are activated by stress (osmotic shock, heat shock, H_2O_2) in *Drosophila* cells (126, 130). Surprisingly, however, the $dMAPK^{p38}$ do not positively regulate immunity signaling in contrast to what appears to be the case in mammalian cells; rather, the $dMAPK^{p38}$ seem to act as attenuators of immunity gene expression (i.e., repression of transcription of the antimicrobial peptides attacin and acropin) (130). This attenuation of immunity gene expression only occurs at late stages of infection and could represent a control mechanism that prevents overstimulation of the immune system and its associated detrimental effects.

In conclusion, the three types of MAPK pathways found in mammalian cells ($MAPK^{erk}$, $MAPK^{jnk}$, and $MAPK^{p38}$ pathways) appear to be present in insect cells. The *Drosophila* system will undoubtly be useful to assess the roles of MAPK pathways, since genetic studies are relatively easily performed in the fruit fly.

D. *Xenopus laevis*

G_2 arrest of *Xenopus* oocytes requires that preformed cyclin B-cdc2 complexes [prematuration-promoting factor (MPF)] be kept in an inactive form that is largely due to inhibitory phosphorylation of MPF. $xMAPK^{erk}$ downregulates the mechanism that inactivates cyclin B-cdc2 kinase and may thus be involved in the exit of the G2 cell cycle block (2). *Xenopus* $MKKK^{mos}$ ($xMAPKKK^{mos}$) is a germ-cell-specific protein that is absent from immature oocytes and is synthesized from stored maternal mRNA in response to progesterone. Translation of $xMKKK^{mos}$ is necessary for progesterone- and insulin-induced maturation of oocytes. $xMKKK^{mos}$ is the only protein that must be synthesized to initiate maturation. $xMKKK^{mos}$ induces rapid activation of MKK and MAPK in oocytes as well as during $xMKKK^{mos}$-induced mitotic arrest in early embryos (276). $xMKKK^{mos}$ action on oocyte maturation may require the *Xenopus* $MKKK^{raf1}$, but probably not in a direct manner, since $MKKK^{raf1}$ activation occurs only several hours after $xMKKK^{mos}$ expression (254).

The spindle assembly checkpoint prevents cells whose spindles are defective or chromosomes are misaligned from initiating anaphase and leaving mitosis. Spindle assembly checkpoint requires the activity of the Ras-dependent MAPK pathway employing the *Xenopus* $MAPK^{erk1}$ and $MAPK^{erk2}$ proteins (331, 356). In contrast, this MAPK pathway appears dispensable for the normal M phase entry and exit (331, 356). In the developing *Xenopus* embryo, the Ras-dependent MAPK pathway is also involved in mesoderm induction (342).

The *Xenopus* system provides a good example of the involvement of MAPK kinase pathways in the regulation of the cell cycle and may prove to be particularly useful in studying the role of MAPK pathways in development.

E. MAPK Pathways in Plants

Stress such as drought, cold, wounding, or pathogen attacks activate MAPK in several plant species and mediates the appropriate defense or survival mechanisms (166, 208, 310, 399). Mechanical stress in the form of touch, rain, and wind also activate MAPK (33).

Auxins are phytohormones that control plant growth

and development. Kinases able to phosphorylate and activate ntMAPK^{mpk2} are stimulated in tobacco cells incubated with auxins (244). This suggests that growth and/or development is also controlled, at least in part, by MAPK pathways.

The increasing number of plant MAPK pathways members that are being characterized (Table 1) indicates that plant MAPK pathways mediate many different aspects of plant physiology. It is also anticipated that there is a great degree of conservation between the plant and the mammalian MAPK pathways, as indicated, for example, by the fact that MAPK pathways transduce stress signals in both types of organisms.

VII. CONCLUDING REMARKS

Saccharomyces cerevisiae, a single-cell eukaryote, has six MAPK, four MKK, and five MKKK encoded in its genome. The corresponding numbers in higher eukaryotes are larger, obviously reflecting the increased complexity of these organisms. For example, the number of characterized MAPK is 12 in mammals, and this will almost certainly increase when the human genome is completely sequenced. The number of MKKK appears to be equal to or larger than that of the MAPK. Why have so many MKKK? The most likely explanation is that different MKKK are integrated into different MAPK modules for responsiveness to different upstream signals such as those originating from cytokines, hormones, cell-cell interactions, and different stress responses (heat, changes in osmolarity, etc.). It is also likely that not only will specific MKKK function in more than one MAPK module, but they will probably have substrates other than MKK for regulation of cell physiology. MEKK1 is a good example, being capable of regulating the MAPK^{jnk} pathway and the NFκB pathway.

What lies ahead in the discovery of MAPK pathways? Obviously, the genome projects for humans, *C. elegans*, and *D. melanogaster* will be enormously insightful. The number of MAPK, MKK, and MKKK in an organism will be predicted from this information. Selective expression of specific MAPK module kinases in given cell types will also be informative. The difficult task will be to characterize functionally and biochemically all the different MAPK modules operating in a cell. The biochemical characterization should determine which MAPK, MKK, and MKKK is used for a given MAPK module, and the functional characterization should define the upstream inputs and downstream substrates for each MAPK module. In addition to biochemistry and experiments employing transfection protocols, genetic approaches, such as gene-targeted disruption (knockouts), will have to be taken to unequivocally define the physiological functions of MAPK pathways. Apparent redundancy in function, particularly at the level of MKKK, makes this task difficult but also extremely important. Finally, the subcellular location of specific MAPK modules is predicted to be a major regulatory property for their regulation. The different structural motifs found in the MKKK suggest different protein-protein interactions for their regulation. These motifs include leucine zippers, proline-rich sequences, 14–3-3 interaction sites, GTP-binding protein interactions, serine/threonine and tyrosine phosphorylation sites, and more. All of these regulatory sites can influence the subcellular location, in addition to the activity of the kinases. Our prediction is that specific MAPK modules will be shown to function as sensors localized in specific regions of the cell to respond to defined extracellular and intracellular imputs for the control of gene expression, metabolism, and the cytoskeleton.

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REFERENCES

1. ABE, J., M. KUSUHARA, R. J. ULEVITCH, B. C. BERK, AND J. D. LEE. Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. *J. Biol. Chem.* 271: 16586–16590, 1996.
2. ABRIEU, A., M. DOREE, AND A. PICARD. Mitogen-activated protein kinase activation down-regulates a mechanism that inactivates cyclin B-cdc2 kinase in G_2-arrested oocytes. *Mol. Biol. Cell* 8: 249–261, 1997.
3. AFTI, A., M. BUISINE, A. MAZARS, AND C. GESPACH. Induction of apoptosis by DPC4, a transcriptional factor regulated by transforming growth factor-β through stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) signaling pathway. *J. Biol. Chem.* 272: 24731–24734, 1997.
4. AHN, N. G., R. SEGER, R. L. BRATLIEN, C. D. DILTZ, N. K. TONKS, AND E. G. KREBS. Multiple components in an epidermal growth factor-stimulated protein kinase cascade. In vitro activation of a myelin basic protein/microtubule-associated protein 2 kinase. *J. Biol. Chem.* 266: 4220–4227, 1991.
5. AITKEN, A., D. JONES, Y. SONEJI, AND S. HOWELL. 14-3-3 Proteins: biological function and domain structure. *Biochem. Soc. Trans.* 23: 605–611, 1995.
6. ALVAREZ, E., I. C. NORTHWOOD, F. A. GONZALEZ, D. A. LATOUR, A. SETH, C. ABATE, T. CURRAN, AND R. J. DAVIS. Pro-Leu-Ser/Thr-Pro is a consensus primary sequence for substrate protein phosphorylation. Characterization of the phosphorylation of c-*myc* and c-*jun* proteins by an epidermal growth factor receptor threonine 669 protein kinase. *J. Biol. Chem.* 266: 15277–15285, 1991.
7. ANDERSON, N. G., J. L. MALLER, N. K. TONKS, AND T. W. STURGILL. Requirement for integration of signals from two distinct phosphorylation pathways for activation of MAP kinase. *Nature* 343: 651–653, 1990.
8. AOKI, M., T. AKIYAMA, J. MIYOSHI, AND K. TOYOSHIMA. Identification and characterization of protein products of the *cot* oncogene with serine kinase activity. *Oncogene* 6: 1515–1519, 1991.
9. AOKI, M., F. HAMADA, T. SUGIMOTO, S. SUMIDA, T. AKIYAMA, AND K. TOYOSHIMA. The human *cot* proto-oncogene encodes two protein serine/threonine kinases with different transforming activities by alternative initiation of translation. *J. Biol. Chem.* 268: 22723–22732, 1993.
10. ASHWORTH, A., S. NAKIELNY, P. COHEN, AND C. MARSHALL. The amino acid sequence of a mammalian MAP kinase kinase. *Oncogene* 7: 2555–2556, 1992.
11. BAGRODIA, S., B. DERIJARD, R. J. DAVIS, AND R. A. CERIONE. Cdc42 and PAK-mediated signaling leads to Jun kinase and p38

mitogen-activated protein kinase activation. *J. Biol. Chem.* 270: 27995–27998, 1995.
12. BANNO, H., K. HIRANO, T. NAKAMURA, K. IRIE, S. NOMOTO, K. MATSUMOTO, AND Y. MACHIDA. NPK1, a tobacco gene that encodes a protein with a domain homologous to yeast BCK1, STE11, and Byr2 protein kinases. *Mol. Cell. Biol.* 13: 4745–4752, 1993.
13. BARDWELL, L., J. G. COOK, E. C. CHANG, B. R. CAIRNS, AND J. THORNER. Signaling in the yeast pheromone response pathway: specific and high-affinity interaction of the mitogen-activated protein (MAP) kinases Kss1 and Fus3 with the upstream MAP kinase kinase Ste7. *Mol. Cell. Biol.* 16: 3637–3650, 1996.
14. BARLOW, P. N., B. LUISI, A. MILNER, M. ELLIOTT, AND R. EVERETT. Structure of the C_3HC_4 domain by ^{1}H-nuclear magnetic resonance spectroscopy. A new structural class of zinc-finger. *J. Mol. Biol.* 237: 201–211, 1994.
15. BASU, T., P. H. WARNE, AND J. DOWNWARD. Role of Shc in the activation of Ras in response to epidermal growth factor and nerve growth factor. *Oncogene* 9: 3483–3491, 1994.
16. BECK, T. W., M. HULEIHEL, M. GUNNELL, T. I. BONNER, AND U. R. RAPP. The complete coding sequence of the human A-raf-1 oncogene and transforming activity of a human A-raf carrying retrovirus. *Nucleic Acids Res.* 15: 595–609, 1997.
17. BEITEL, G. J., S. TUCK, I. GREENWALD, AND H. R. HORVITZ. The *Caenorhabditis elegans* gene *lin-1* encodes an ETS-domain protein and defines a branch of the vulval induction pathway. *Genes Dev.* 9: 3149–3162, 1995.
18. BIENSTOCK, R. J., T. DARDEN, R. WISEMAN, L. PEDERSEN, AND J. C. BARRETT. Molecular modeling of the amino-terminal zinc Ring domain of BRCA1. *Cancer Res.* 56: 2539–2545, 1996.
19. BIGGS, W. H., III, K. H. ZAVITZ, B. DICKINSON, A. VAN DER STRATEN, D. BRUNNER, E. HAFEN, AND S. L. ZIPURSKY. The *Drosophila* rolled locus encodes a MAP kinase required in the sevenless signal transduction pathway. *EMBO J.* 13: 1628–1635, 1994.
20. BLANK, J. L., P. GERWINS, E. M. ELLIOTT, S. SATHER, AND G. L. JOHNSON. Molecular cloning of mitogen-activated protein/ERK kinase kinases (MEKK) 2 and 3. Regulation of sequential phosphorylation pathways involving mitogen-activated protein kinase and c-Jun kinase. *J. Biol. Chem.* 271: 5361–5368, 1996.
21. BLUMER, K. J., AND G. L. JOHNSON. Diversity in function and regulation of MAP kinase pathways. *Trends Biochem. Sci.* 19: 236–240, 1994.
22. BOGOYEVITCH, M. A., C. J. MARSHALL, AND P. H. SUGDEN. Hypertrophic agonists stimulate the activities of the protein kinases c-Raf and A-Raf in cultured ventricular myocytes. *J. Biol. Chem.* 270: 26303–26310, 1995.
23. BOGUSLAWSKI, G. PBS2, a yeast gene encoding a putative protein kinase, interacts with the RAS2 pathway and affects osmotic sensitivity of *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* 138: 2425–2432, 1992.
24. BOGUSLAWSKI, G., AND J. O. POLAZZI. Complete nucleotide sequence of a gene conferring polymyxin B resistance on yeast: similarity of the predicted polypeptide to protein kinases. *Proc. Natl. Acad. Sci. USA* 84: 5848–5852, 1987.
25. BOKEMEYER, D., A. SOROKIN, M. YAN, N. G. AHN, D. J. TEMPLETON, AND M. J. DUNN. Induction of mitogen-activated protein kinase phosphatase 1 by the stress-activated protein kinase signaling pathway but not by extracellular signal-regulated kinase in fibroblasts. *J. Biol. Chem.* 271: 639–642, 1996.
26. BONNER, T. I., H. OPPERMANN, P. SEEBURG, S. B. KERBY, M. A. GUNNELL, A. C. YOUNG, AND U. R. RAPP. The complete coding sequence of the human *raf* oncogene and the corresponding structure of the *c-raf*-1 gene. *Nucleic Acids Res.* 14: 1009–1015, 1986.
27. BONNY, C., P. NICOD, AND G. WAEBER. IB1, a JIP-1-related nuclear protein present in insulin-secreting cells. *J. Biol. Chem.* 273: 1843–1846, 1998.
28. BORDEN, K. L., M. N. BODDY, J. LALLY, N. J. O'REILLY, S. MARTIN, K. HOWE, E. SOLOMON, AND P. S. FREEMONT. The solution structure of the RING finger domain from the acute promyelocytic leukaemia proto-oncoprotein PML. *EMBO J.* 14: 1532–1541, 1995.
29. BORDEN, K. L., AND P. S. FREEMONT. The RING finger domain: a recent example of a sequence-structure family. *Curr. Opin. Struct. Biol.* 6: 395–401, 1996.
30. BORNFELDT, K. E., J. S. CAMPBELL, H. KOYAMA, G. M. ARGAST, C. C. LESLIE, E. W. RAINES, E. G. KREBS, AND R. ROSS. The mitogen-activated protein kinase pathway can mediate growth inhibition and proliferation in smooth muscle cells. Dependence on the availability of downstream targets. *J. Clin. Invest.* 100: 875–885, 1997.
31. BOULTON, T. G., S. H. NYE, D. J. ROBBINS, I. NY, E. RADZIEJEWSKA, S. D. MORGENBESSER, R. A. DEPINHO, N. PANAYOTATOS, M. H. COBB, AND G. D. YANCOPOULOS. ERKS: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* 65: 663–675, 1991.
32. BOULTON, T. G., G. D. YANCOPOULOS, J. S. GREGORY, C. SLAUGHTER, C. MOOMAW, J. HSU, AND M. H. COBB. An insulin-stimulated protein kinase similar to yeast kinases involved in cell cycle control. *Science* 249: 64–67, 1990.
33. BOĞRE, L., W. LIGTERINK, E. HEBERLE-BORS, AND H. HIRT. Mechanosensors in plants. *Nature* 383: 489–490, 1996.
34. BRENNER, B., U. KOPPENHOEFER, C. WEINSTOCK, O. LINDERKAMP, F. LANG, AND E. GULBINS. Fas- or ceramide-induced apoptosis is mediated by a Rac1-regulated activation of Jun N-terminal kinase/p38 kinases and GADD153. *J. Biol. Chem.* 272: 22173–22181, 1997.
35. BREWSTER, J. L., T. DE VALOIR, N. D. DWYER, E. WINTER, AND M. C. GUSTIN. An osmosensing signal transduction pathway in yeast. *Science* 259: 1760–1763, 1993.
36. BRIZA, P., A. ELLINGER, G. WINKLER, AND M. BREITENBACH. Chemical composition of the yeast ascospore wall. The second outer layer consists of chitosan. *J. Biol. Chem.* 263: 11569–11574, 1988.
37. BRIZA, P., A. ELLINGER, G. WINKLER, AND M. BREITENBACH. Characterization of a DL-dityrosine-containing macromolecule from yeast ascospore walls. *J. Biol. Chem.* 265: 15118–15123, 1990.
38. BROTT, B. K., A. ALESSANDRINI, D. A. LARGAESPADA, N. G. COPELAND, N. A. JENKINS, C. M. CREWS, AND R. L. ERIKSON. MEK2 is a kinase related to MEK1 and is differentially expressed in murine tissues. *Cell Growth Differ.* 4: 921–929, 1994.
39. CALOGERAKI, I., J. V. BARNIER, A. EYCHENE, M. P. FELDER, G. CALOTHY, AND M. MARX. Genomic organization and nucleotide sequence of the coding region of teh chicken c-Rmil (B-raf-1) proto-oncogene. *Biochem. Biophys. Res. Commun.* 193: 1324–1331, 1993.
40. CANAGARAJAH, B. J., A. KHOKHLATCHEV, M. H. COBB, AND E. J. GOLDSMITH. Activation mechanism of the MAP kinase ERK2 by dual phosphorylation. *Cell* 90: 859–869, 1997.
41. CARDONE, M., G. S. SALVESEN, C. WIDMANN, G. L. JOHNSON, AND S. M. FRISCH. The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. *Cell* 90: 315–323, 1997.
42. CASILLAS, A. M., K. AMARAL, S. CHEGINI-FARAHANI, AND A. E. NEL. Okadaic acid activates p42 mitogen-activated protein kinase (MAP kinase; ERK-2) in B-lymphocytes but inhibits rather than augments cellular proliferation: contrast with phorbol 12-myristate 13-acetate. *Biochem. J.* 290: 545–550, 1993.
43. CATLING, A. D., C. W. REUTER, M. E. COX, S. J. PARSONS, AND M. J. WEBER. Partial purification of a mitogen-activated protein kinase kinase activator from bovine brain. Identification as B-Raf or a B-Raf-associated activity. *J. Biol. Chem.* 269: 30014–30021, 1994.
44. CECI, J. D., C. P. PATRIOTIS, C. TSATSANIS, A. M. MAKRIS, R. KOVATCH, D. A. SWING, N. A. JENKINS, P. N. TSICHLIS, AND N. G. COPELAND. Tpl-2 is an oncogenic kinase that is activated by carboxy-terminal truncation. *Genes Dev.* 11: 688–700, 1997.
45. CHALEFF, D. T., AND K. TATCHELL. Molecular cloning and characterization of the STE7 and STE11 genes of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 5: 1878–1886, 1985.
46. CHAN, A. C., AND A. S. SHAW. Regulation of antigen receptor signal transduction by protein tyrosine kinases. *Curr. Opin. Immunol.* 8: 394–401, 1996.
47. CHAN, E. D., B. W. WINSTON, M. B. JARPE, M. W. WYNES, AND D. W. H. RICHES. Preferential activation of the p46 isoform of JNK/SAPK in mouse macrophage by TNFα. *Proc. Natl. Acad. Sci. USA* 94: 13169–13174, 1997.
48. CHANG, E. C., M. BARR, Y. WANG, V. JUNG, H. P. XU, AND M. H. WIGLER. Cooperative interaction of *S. pombe* proteins required for mating and morphogenesis. *Cell* 79: 131–141, 1994.
49. CHAREST, D. L., G. MORDRET, K. W. HARDER, F. JIRIK, AND S. L. PELECH. Molecular cloning, expression, and characterization of

the human mitogen-activated protein kinase p44erk1. *Mol. Cell. Biol.* 13: 4679–4690, 1993.
50. CHEN, Q., M. S. KINCH, T. H. LIN, K. BURRIDGE, AND R. L. JULIANO. Integrin-mediated cell adhesion activates mitogen-activated protein kinases. *J. Biol. Chem.* 269: 26602–26605, 1994.
51. CHEN, Y.-R., X. WANG, D. TEMPLETON, R. J. DAVIS, AND T.-H. TAN. The role of c-Jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C and gamma irradiation. Duration of JNK activation may determine cell death and proliferation. *J. Biol. Chem.* 271: 31929–31936, 1996.
52. CHENG, M., T. G. BOULTON, AND M. H. COBB. ERK3 is a constitutively nuclear protein kinase. *J. Biol. Chem.* 271: 8951–8958, 1996.
53. CHOI, K. Y., B. SATTERBERG, D. M. LYONS, AND E. A. ELION. Ste5 tethers multiple protein kinase in the MAP kinase cascade required for mating in *S. cerevisiae*. *Cell* 78: 499–512, 1994.
54. CHOW, C. W., M. RINCON, J. CAVANAGH, M. DICKENS, AND R. J. DAVIS. Nuclear accumulation of NFAT4 opposed by the JNK signal transduction pathway. *Science* 278: 1638–1641, 1997.
55. CHU, Y., P. A. SOLSKI, R. KHOSRAVI-FAR, C. J. DER, AND K. KELLY. The mitogen-activated protein kinase phosphatases PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity in vivo toward the ERK2 sevenmaker mutation. *J. Biol. Chem.* 271: 6497–6501, 1996.
56. CONKLIN, D. S., K. GALAKTIONOV, AND D. BEACH. 14-3-3 Proteins associate with cdc25 phosphatases. *Proc. Natl. Acad. Sci. USA* 92: 7892–7896, 1995.
57. COOPER, J. A. Related proteins are phosphorylated at tyrosine in response to mitogenic stimuli and at meiosis. *Mol. Cell. Biol.* 9: 3143–3147, 1989.
58. COOPER, J. A. Straight and narrow or tortuous and intersecting? *Curr. Biol.* 4: 1118–1121, 1994.
59. COOPER, J. A., D. F. BOWEN-POPE, E. RAINES, R. ROSS, AND T. HUNTER. Similar effects of platelet-derived growth factor and epidermal growth factor on the phosphorylation of tyrosine in cellular proteins. *Cell* 31: 263–273, 1982.
60. COOPER, J. A., AND T. HUNTER. Changes in protein phosphorylation in Rous sarcoma virus-transformed chicken embryo cells. *Mol. Cell. Biol.* 1: 165–178, 1981.
61. COOPER, J. A., AND T. HUNTER. Identification and characterization of cellular targets for tyrosine protein kinases. *J. Biol. Chem.* 258: 1108–1115, 1983.
62. COSO, O. A., M. CHIARIELLO, J.-C. YU, H. TERAMOTO, P. CRESPO, N. XU, T. MIKI, AND J. S. GUTKIND. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 81: 1137–1146, 1995.
63. COURCHESNE, W. E., R. KUNISAWA, AND J. THORNER. A putative protein kinase overcomes pheromone-induced arrest of cell cycling in *S. cerevisiae*. *Cell* 58: 1107–1119, 1989.
64. CRAWLEY, J. B., L. RAWLINSON, F. V. LALI, T. H. PAGE, J. SAKLATVALA, AND B. M. FOXWELL. T cell proliferation in response to interleukins 2 and 7 requires p38MAP kinase activation. *J. Biol. Chem.* 272: 15023–15027, 1997.
65. CREWS, C. M., A. A. ALESSANDRINI, AND R. L. ERIKSON. Mouse Erk-1 gene product is a serine/threonine protein kinase that has the potential to phosphorylate tyrosine. *Proc. Natl. Acad. Sci. USA* 88: 8845–8849, 1991.
66. CREWS, C. M., A. ALESSANDRINI, AND R. L. ERIKSON. The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. *Science* 258: 478–480, 1992.
67. CREWS, C. M., AND R. L. ERIKSON. Extracellular signals and reversible protein phosphorylation: what to Mek of it all. *Cell* 74: 215–217, 1993.
68. CUENDA, A., G. ALONSA, N. MORRICE, M. JONES, R. MEIER, P. COHEN, AND A. R. NEBREDA. Purification and cDNA cloning of SAPKK3, the major activator of RK/p38 in stress- and cytokine-stimulated monocytes and epithelial cells. *EMBO J.* 15: 4156–4164, 1996.
69. DAUB, H., F. U. WEISS, C. WALLASCH, AND A. ULLRICH. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* 379: 557–560, 1996.
70. DECROOCQ-FERRANT, V., S. DECROOCQ, J. VAN WENT, E. SCHMIDT, AND M. KREIS. A homologue of the MAP/ERK family of protein kinase genes is expressed in vegetative and in female reproductive organs of *Petunia hybrida*. *Plant Mol. Biol.* 27: 339–350, 1995.
71. DE MIGUEL, C., D. KLIGMAN, J. PATEL, AND S. D. DETERA-WADLEIGH. Molecular analysis of microtubule-associated protein-2 kinase cDNA from mouse and rat brain. *DNA Cell Biol.* 10: 505–514, 1991.
72. DENT, P., T. JELINEK, D. K. MORRISON, M. J. WEBER, AND T. W. STURGILL. Reversal of Raf-1 activation by purified and membrane-associated protein phosphatases. *Science* 268: 1902–1906, 1995.
73. DERIJARD, B., M. HIBI, I. WU, T. BARRETT, B. SU, T. DENG, M. KARIN, AND R. J. DAVIS. JNK-1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-*jun* activation domain. *Cell* 76: 1025–1037, 1994.
74. DERIJARD, B., J. RAINGEAUD, T. BARRETT, I.-H. WU, J. HAN, R. J. ULEVITCH, AND R. J. DAVIS. Independent human MAP kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 267: 682–685, 1995.
75. DICKENS, M., J. S. ROGERS, J. CAVANAGH, A. RAITANO, Z. XIA, J. R. HALPERN, M. E. GREENBERG, C. L. SAWYERS, AND R. J. DAVIS. A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* 277: 693–696, 1997.
76. DIKIC, I., J. SCHLESSINGER, AND I. LAX. PC12 cells overexpressing the insulin receptor undergo insulin-dependent neuronal differentation. *Curr. Biol.* 4: 702–708, 1994.
77. DOHLMAN, H. G., AND J. THORNER. RGS proteins and signaling by heterotrimeric G proteins. *J. Biol. Chem.* 272: 3871–3874, 1997.
78. DORING, F., G. DREWES, B. BERLING, AND E. M. MANDELKOW. Cloning and sequencing of a cDNA encoding rat brain mitogen-activated protein (MAP) kinase activator. *Gene* 131: 303–304, 1993.
79. DOROW, D. S., L. DEVEREUX, G. F. TU, G. PRICE, J. K. NICHOLL, G. R. SUTHERLAND, AND R. J. SIMPSON. Complete nucleotide sequence, expression, and chromosomal localisation of human mixed-lineage kinase 2. *Eur. J. Biochem.* 234: 492–500, 1995.
80. DOWNWARD, J. Control of ras activation. *Cancer Surv.* 27: 87–100, 1996.
81. DUERR, B., M. GAWIENOWSKI, T. ROPP, AND T. JACOBS. MsERK1: a mitogen-activated protein kinase from a flowering plant. *Plant Cell* 5: 87–96, 1993.
82. ELDAR-FINKELMAN, H., R. SEGER, J. R. VANDENHEEDE, AND E. G. KREBS. Inactivation of glycogen synthase kinase-3 by epidermal growth factor is mediated by mitogen-activated protein kinase/p90 ribosomal protein S6 kinase signaling pathway in NIH/3T3 cells. *J. Biol. Chem.* 270: 987–990, 1995.
83. ELION, E. A., J. A. BRILL, AND G. R. FINK. FUS3 represses CLN1 and CLN2 and in concert with KSS1 promotes signal transduction. *Proc. Natl. Acad. Sci. USA* 88: 9392–9396, 1991.
84. ELION, E. A., P. L. GRISAFI, AND G. R. FINK. FUS3 encodes a cdc2+/CDC28-related kinase required for the transition from mitosis into conjugation. *Cell* 60: 649–664, 1990.
85. ELION, E. A., B. SATTERBERG, AND J. E. KRANZ. FUS3 phosphorylates multiple components of the mating signal transduction cascade: evidence for STE12 and FAR1. *Mol. Biol. Cell* 4: 495–510, 1993.
86. ELLINGER-ZIEGELBAUER, H., K. BROWN, K. KELLY, AND U. SIEBENLIST. Direct activation of the stress-activated protein kinase (SAPK) and extracellular signal-regulated protein kinase (ERK) pathways by an inducible mitogen-activated protein kinase/ERK kinase kinase 3 (MEKK) derivative. *J. Biol. Chem.* 272: 2668–2674, 1997.
87. ENGLISH, J. D., AND J. D. SWEATT. Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. *J. Biol. Chem.* 271: 24329–24332, 1996.
88. ENGLISH, J. M., C. A. VANDERBILT, S. XU, S. MARCUS, AND M. H. COBB. Isolation of MEK5 and differential expression of alternatively spliced forms. *J. Biol. Chem.* 270: 28897–28902, 1995.
89. ERREDE, B., AND D. E. LEVIN. A conserved kinase cascade for MAP kinase activation in yeast. *Curr. Opin. Cell Biol.* 5: 254–260, 1993.
90. ERSHLER, M., T. V. NAGORSKAYA, J. W. VISSER, AND A. V. BELYAVSKY. Novel cdc2-related protein kinase produced in murine hematopoietic stem cells. *Gene* 124: 305–306, 1993.
91. EYCHENE, A., J. V. BARNIER, P. DEZELEE, M. MARX, D. LAUGIER, I. CALOGERAKI, AND G. CALOTHY. Quail neurotina c-Rmil(B-raf) proto-oncogene cDNAs encode two proteins of 93.5

and 95 kDa resulting from alternative splicing. *Oncogene* 7: 1315–1323, 1992.
92. FANGER, G. R., P. GERWINS, C. WIDMANN, M. B. JARPE, AND G. L. JOHNSON. MEKKs, GCKs, MLKs, PAKs, TAKs, and Tpls: upstream regulators of the c-Jun amino-terminal kinases? *Curr. Opin. Genet. Dev.* 7: 67–74, 1997.
93. FANGER, G. R., N. LASSIGNAL JOHNSON, AND G. L. JOHNSON. MEK kinases are regulated by EGF and selectively interact with Rac/Cdc42. *EMBO J.* 16: 4961–4972, 1997.
94. FANGER, G. R., C. WIDMANN, A. C. PORTER, S. SATHER, G. L. JOHNSON, AND R. R. VAILLANCOURT. 14-3-3 Proteins interact with specific MEK kinases. *J. Biol. Chem.* 273: 3476–3483, 1998.
95. FANTL, W. J., A. J. MUSLIN, A. KIKUCHI, J. A. MARTIN, A. M. MACNICOL, R. W. GROSS, AND L. T. WILLIAMS. Activation of Raf-1 by 14-3-3 proteins. *Nature* 371: 612–614, 1994.
96. FARIS, M., N. KOKO, L. LEE, AND A. E. NEL. Regulation of interleukin-2 transcription by inducible stable expression of dominant negative and dominant active mitogen-activated protein kinase kinase kinase in Jurkat T cells. Evidence for the importance of Ras in a pathway that is controlled by dual receptor stimulation. *J. Biol. Chem.* 271: 27366–27373, 1996.
97. FARIS, M., N. KOKOT, K. LATINIS, S. KASIBHATLA, D. R. GREEN, G. A. KORETZKY, AND A. NEL. The c-Jun N-terminal kinase cascade plays a role in stress-induced apoptosis in Jurkat cells by up-regulating Fas ligand expression. *J. Immunol.* 160: 134–144, 1998.
98. FAUX, M. C., AND J. D. SCOTT. More on target with protein phosphorylation: conferring specificity by location. *Trends Biochem. Sci.* 21: 312–315, 1996.
99. FORCE, T., J. V. BONVENTRE, G. HEIDECKER, U. RAPP, J. AVRUCH, AND J. M. KYRIAKIS. Enzymatic characteristics of the c-Raf-1 protein kinase. *Proc. Natl. Acad. Sci. USA* 91: 1270–1274, 1994.
100. FRANKLIN, C. C., AND A. S. KRAFT. Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells. *J. Biol. Chem.* 272: 16917–16923, 1997.
101. FREED, E., M. SYMONS, S. G. MACDONALD, F. MCCORMICK, AND R. RUGGIERI. Binding of 14-3-3 proteins to the protein kinase Raf and effects on its activation. *Science* 265: 1713–1716, 1994.
102. FREEMAN, R. S., K. M. PICKHAM, J. P. KANKI, B. A. LEE, S. V. PENA, AND D. J. DONOGHUE. *Xenopus* homolog of the *mos* protooncogene transforms mammalian fibroblasts and induces maturation of *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* 86: 5805–5809, 1989.
103. FU, H., K. XIA, D. C. PALLAS, C. CUI, K. CONROY, R. P. NARSIMHAN, H. MAMON, R. J. COLLIER, AND T. M. ROBERTS. Interaction of the protein kinase Raf-1 with 14-3-3 proteins. *Science* 266: 126–129, 1994.
104. FUKUDA, M., I. GOTOH, M. ADACHI, Y. GOTOH, AND E. NISHIDA. A novel regulatory mechanism in the mitogen-activated protein (MAP) kinase cascade. Role of nuclear export signal of map kinase kinase. *J. Biol. Chem.* 272: 32642–32648, 1997.
105. GALCHEVA-GARGOVA, Z., B. DERIJARD, I. H. WU, AND R. J. DAVIS. An osmosensing signal transduction pathway in mammalian cells. *Science* 265: 806–808, 1994.
106. GALLO, K. A., M. R. MARK, D. T. SCADDEN, Z. WANG, Q. GU, AND P. J. GODOWSKI. Identification and characterization of SPRK, a novel src-homology 3 domain-containing proline-rich kinase with serine/threonine kinase activity. *J. Biol. Chem.* 269: 15092–15100, 1994.
107. GARCIA, J. I., G. ZALBA, S. D. DETERA-WADLEIGH, AND C. DE MIGUEL. Isolation of a cDNA encoding the rat MAP-kinase homolog of human p63mapk. *Mamm. Genome* 7: 810–814, 1996.
108. GARDNER, A. M., AND G. L. JOHNSON. Fibroblast growth factor-2 suppression of tumor necrosis factor α-mediated apoptosis requires ras and the activation of mitogen-activated protein kinase. *J. Biol. Chem.* 271: 14560–14566, 1996.
109. GARTNER, A., K. NASMYTH, AND G. AMMERER. Signal transduction in *Saccharomyces cerevisiae* requires tyrosine and threonine phosphorylation of FUS3 and KSS1. *Genes Dev.* 6: 1280–1292, 1992.
110. GASKINS, C., M. MAEDA, AND R. A. FIRTEL. Identification and funtional analysis of a developmentally regulated extracellular signal-regulated kinase gene in *Dictyostelium discoideum*. *Mol. Cell. Biol.* 14: 6996–7012, 1994.
111. GERWINS, P., J. L. BLANK, AND G. L. JOHNSON. Cloning of a novel mitogen-activated protein kinase kinase kinase, MEKK4, that selectively regulates the c-Jun amino terminal kinase pathway. *J. Biol. Chem.* 272: 8288–8295, 1997.
112. GIMENO, C. J., AND G. R. FINK. The logic of cell division in the life cycle of yeast. *Science* 257: 626, 1992.
113. GIMENO, C. J., P. O. LJUNGDAHL, C. A. STYLES, AND G. R. FINK. Unipolar cell division in the yeast *S. cerevisiae* leads to filamentous growth: regulation by starvation and RAS. *Cell* 68: 1077–1090, 1992.
114. GLISE, B., H. BOURBON, AND S. NOSELLI. Hemipterous encodes a novel *Drosophila* MAP kinase kinase, required for epithelial cell sheet movement. *Cell* 83: 451–461, 1995.
115. GOEDERT, M., A. CUENDA, M. CRAXTON, R. JAKES, AND P. COHEN. Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6): comparison of its substrate specificity with that of other SAP kinases. *EMBO J.* 16: 3563–3571, 1997.
116. GOEDERT, M., J. HASEGAWA, M. CRAXTON, M. A. LEVERSHA, AND S. CLEGG. Assignment of the human stress-activated protein kinase-3 gene (SAPK3) to chromosome 22q13.3 by fluorescence in situ hybridization. *Genomics* 41: 501–502, 1997.
117. GOILLOT, E., J. RAINGEAUD, A. RANGER, R. I. TEPPER, R. J. DAVIS, E. HARLOW, AND I. SANCHEZ. Mitogen-activated protein kinase-mediated Fas apoptotic signaling pathway. *Proc. Natl. Acad. Sci. USA* 94: 3302–3307, 1997.
118. GONZALEZ, F. A., D. L. RADEN, M. R. RIGBY, AND R. J. DAVIS. Heterogeneous expression of four AMP kinase isoforms in human tissues. *FEBS Lett.* 304: 170–178, 1992.
119. GORDON, R. D., I. A. LEIGHTON, D. G. CAMPBELL, P. COHEN, A. CREANY, D. C. WILTON, D. J. MASTERS, G. A. RITCHIE, R. MOTT, I. W. TAYLOR, K. R. BUNDELL, L. DOUGLAS, J. MORTEN, AND M. NEEDHAM. Cloning and expression of cystolic phospholipase A_2 ($cPLA_2$) and a naturally occurring variant. Phosphorylation of Ser505 of recombinant $cPLA_2$ by p42 mitogen-activated protein kinase results in an increase in specific activity. *Eur. J. Biochem.* 238: 690–697, 1996.
120. GOTOH, Y., K. MORIYAMA, S. MATSUDA, E. OKUMURA, T. KISHIMOTO, H. KAWASAKI, K. SUZUKI, I. YAHARA, H. SAKAI, AND E. NISHIDA. *Xenopus* M phase MAP kinase: isolation of its cDNA and activation by MPF. *EMBO J.* 10: 2661–2668, 1991.
121. GOWEN COOK, J., L. BARDWELL, AND J. THORNER. Inhibitory and activating functions for MAPK Kss1 in the *S. cerevisiae* filamentous-growth signalling pathway. *Nature* 390: 85–88, 1997.
122. GROOM, L. A., A. A. SNEDDON, D. R. ALESSI, S. DOWD, AND S. M. KEYSE. Differential regulation of the MAP, SAP and RK/p38 kinases by Pyst1, a novel cytosolic dual-specificity phosphatase. *EMBO J.* 15: 3621–3632, 1996.
123. GUPTA, S., T. BARRETT, A. J. WHITMARSH, J. CAVANAGH, H. K. SLUSS, B. DERIJARD, AND R. J. DAVIS. Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J.* 15: 2760–2770, 1996.
124. GUPTA, S., D. CAMPBELL, B. DERIJARD, AND R. J. DAVIS. Transcription factor ATF2 regulation by the JNK signal transduction pathway. *Science* 267: 389–393, 1995.
125. HAM, J., C. BABIJ, J. WHITFIELD, C. M. PFARR, D. LALLEMAND, M. YANIV, AND L. L. RUBIN. A c-Jun dominant negative mutant protects sympathetic neurons against programmed cell death. *Neuron* 14: 927–939, 1995.
126. HAN, J., K. Y. CHOI, P. T. BREY, AND W. J. LEE. Molecular cloning and characterization of a *Drosophila* p38 mitogen-activated protein kinase. *J. Biol. Chem.* 273: 369–374, 1998.
127. HAN, J., Y. JIANG, Z. LI, V. V. KRAVCHENKO, AND R. J. ULEVITCH. Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. *Nature* 386: 296–299, 1997.
128. HAN, J., J. D. LEE, L. BIBBS, AND R. J. ULEVITCH. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 265: 808–811, 1994.
129. HAN, M., A. GOLDEN, Y. HAN, AND P. W. STERNBERG. *C. elegans* lin-45 raf gene participate in let-60 ras-stimulated vulval differentiation. *Nature* 363: 133–140, 1993.
130. HAN, Z. S., H. ENSLEN, X. HU, X. MENG, I. WU, T. BARRETT, R. J. DAVIS, AND Y. T. IP. A conserved p38 mitogen-activated protein kinase pathway regulates *Drosophila* immunity gene expression. *Mol. Cell. Biol.* 18: 3527–3539, 1998.

131. HANCOCK, J. F., K. CADWALLADER, H. PATERSON, AND C. J. MARSHALL. A CAAX or a CAAL motif and a second signal are sufficient for plasma membrane targeting of ras proteins. *EMBO J.* 10: 4033–4039, 1991.
132. HASHIMOTO, H., Y. MATSUO, Y. YOKOYAMA, H. TOYOHARA, AND M. SAKAGUCHI. Structure and expression of carp mitogen-activated protein kinases homologous to mammalian JNK/SAPK. *J. Biochem.* 122: 381–386, 1997.
133. HAYSTEAD, C. M., P. GREGORY, A. SHIRAZI, P. FADDEN, C. MOSSE, P. DENT, AND T. A. HAYSTEAD. Insulin activates a novel adipocyte mitogen-activated protein kinase kinase kinase that shows rapid phasic kinetics and is distinct from c-Raf. *J. Biol. Chem.* 269: 12804–12808, 1994.
134. HER, J. H., J. WU, T. B. RALL, T. W. STURGILL, AND M. J. WEBER. Sequence of pp42/MAP kinase, a serine/threonine kinase regulated by tyrosine phosphorylation. *Nucleic Acids Res.* 19: 37–43, 1991.
135. HERMEKING, H., C. LENGAUER, K. POLYAK, T.-C. HE, L. ZHANG, S. THIAGALINGAM, K. W. KINZLER, AND B. VOGELSTEIN. 14-3-3σ is a p53-regulated inhibitor of G_2/M progression. *Mol. Biol. Cell* 1: 3–11, 1997.
136. HERSKOWITZ, I. MAP kinase pathways in yeast: for mating and more. *Cell* 80: 187–197, 1995.
137. HIRAI, S.-I., M. IZAWA, S.-I. OSADA, G. SPYROU, AND S. OHNO. Activation of the JNK pathway by distantly related protein kinases, MEKK and MUK. *Oncogene* 12: 641–650, 1996.
138. HIRAI, S.-I., M. KATOH, M. TERADA, J. M. KYRIAKIS, L. I. ZON, A. RANA, J. AVRUCH, AND S. OHNO. MST/MLK2, a member of the mixed lineage kinase family, directly phosphorylates and activates SEK1, an activator of c-Jun N-terminal kinase/stress-activated protein kinase. *J. Biol. Chem.* 272: 15167–15173, 1997.
139. HIRANO, M., S.-I. OSADA, T. AOKI, S.-I. HIRAI, M. HOSAKA, J.-I. INOUE, AND S. OHNO. MEK kinase is involved in tumor necrosis factor α-induced NF-kB activation and degradation of IkB-α. *J. Biol. Chem.* 271: 13234–13238, 1996.
140. HIRSCH, D. D., AND P. J. S. STORK. Mitogen-activated protein kinase phosphatases inactivate stress-activated protein kinase pathways in vivo. *J. Biol. Chem.* 272: 4568–4575, 1997.
141. HOLLAND, P. M., M. SUZANNE, J. S. CAMPBELL, S. NOSELLI, AND J. A. COOPER. MKK7 is a stress-activated mitogen-activated protein kinase kinase functionally related to hemipterous. *J. Biol. Chem.* 272: 24994–24998, 1997.
142. HOLZMAN, L. B., S. E. MERRITT, AND G. FAN. Identification, molecular cloning, and characterization of dual leucine zipper bearing kinase. A novel serine/threonine protein kinase that defines a second subfamily of mixed lineage kinases. *J. Biol. Chem.* 269: 30808–30817, 1994.
143. HOU, X. S., E. S. GOLDSTEIN, AND N. PERRIMON. *Drosophila* Jun relays the Jun amino-terminal kinase signal transduction pathway to the Decapentaplegic signal transduction pathway in regulating epithelial cell sheet movement. *Genes Dev.* 11: 1728–1737, 1997.
144. HOWE, L. R., S. J. LEEVERS, N. GOMEZ, S. NAKIELNY, P. COHEN, AND C. J. MARSHALL. Activation of the MAP kinase pathway by the protein kinase raf. *Cell* 71: 335–342, 1992.
145. HU, M. C., W. R. QIU, AND Y. P. WANG. JNK1, JNK2 and JNK3 are p53 N-terminal serine 34 kinases. *Oncogene* 15: 2277–2287, 1997.
146. HUANG, C. J., M. S. LEE, G. D. CHANG, F. L. HUANG, AND T. B. LO. Molecular cloning and sequencing of a carp cDNA encoding mitogen-activated protein kinase kinase. *Biochim. Biophys. Acta* 1220: 223–225, 1994.
147. HUNTER, T., AND G. D. PLOWMAN. The protein kinases of budding yeast: six score and more. *Trends Biochem. Sci.* 22: 18–22, 1997.
148. HUTTLY, A. K., AND A. L. PHILLIPS. Gibberellin-regulated expression in oat aleurone cells of two kinases that show homology to MAP kinase and a ribosomal protein kinase. *Plant Mol. Biol.* 27: 1043–1052, 1995.
149. ICHIJO, H., E. NISHIDA, K. IRIE, P. T. DIJKE, M. SAITOH, T. MORIGUCHI, M. TAKAGI, K. MATSUMOTO, K. MIYAZONO, AND Y. GOTOH. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275: 90–94, 1997.
150. ING, Y. L., I. W. LEUNG, H. H. HENG, L. C. TSUI, AND N. J. LASSAM. MLK-3: identification of a widely-expressed protein kinase bearing an SH3 domain and a leucine zipper-basic region domain. *Oncogene* 9: 1745–1750, 1994.
151. INOUYE, C., N. DHILLON, T. DURFEE, P. C. ZAMBRYSKI, AND J. THORNER. Mutational analysis of STE5 in the yeast *Saccharomyces cerevisiae*: application of a differential interaction trap assay for examining protein-protein interactions. *Genetics* 147: 479–492, 1997.
152. INOUYE, C., N. DHILLON, AND J. THORNER. Ste5 RING-H2 domain: role in Ste4-promoted oligomerization for yeast pheromone signaling. *Science* 278: 103–106, 1997.
153. IRIE, K., Y. GOTOH, B. M. YASHAR, B. ERREDE, E. NISHIDA, AND K. MATSUMOTO. Stimulatory effects of yeast and mammalian 14-3-3 proteins on the Raf protein kinase. *Science* 265: 1716–1719, 1994.
154. IRIE, K., M. TAKASE, K. S. LEE, D. E. LEVIN, H. ARAKI, K. MATSUMOTO, AND Y. OSHIMA. MKK1 and MKK2, which encode *Saccharomyces cerevisiae* mitogen-activated protein kinase-kinase homologs, function in the pathway mediated by protein kinase C. *Mol. Cell. Biol.* 13: 3076–3083, 1993.
155. ISHIKAWA, F., F. TAKAKU, M. NAGAO, AND T. SUGIMURA. Rat *c-raf* oncogene activation by a rearrangement that produces a fused protein. *Mol. Cell. Biol.* 7: 1226-1232, 1987.
156. ISHIKAWA, F., F. TAKAKU, M. NAGAO, AND T. SUGIMURA. The complete primary structure of the rat A-raf cDNA coding region: conservation of the putative regulatory regions present in rat c-raf. *Oncogene Res.* 1: 243–253, 1997.
157. ISHIKAWA, T., Y. NAKADA-MORIYA, C. ANDO, N. TANDA, S. NISHIDA, Y. MINATOGAWA, AND T. NOHNON. Expression of the JNK2-alpha1 gene in the developing chick brain. *Biochem. Biophys. Res. Commun.* 234: 489–492, 1997.
158. JAISWAL, R. K., S. A. MOODIE, A. WOLFMAN, AND G. E. LANDRETH. The mitogen-activated protein kinase cascade is activated by B-Raf in response to nerve growth factor through interaction with p21ras. *Mol. Cell. Biol.* 14: 6944–6953, 1994.
159. JANKNECHT, R., AND T. HUNTER. Activation of the Sap-1a transcription factor by the c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase. *J. Biol. Chem.* 272: 4219–4224, 1997.
160. JARPE, M. B., C. WIDMANN, C. KNALL, T. K. SCHLESINGER, S. GIBSON, T. YUJURI, G. R. FANGER, E. G. GELFAND, AND G. L. JOHNSON. Anti-apoptotic versus pro-apoptotic signal transduction: checkpoints and stop signs along the road to death. *Oncogene.* In press.
161. JELINEK, T., A. D. CATLING, C. W. REUTER, S. A. MOODIE, A. WOLFMAN, AND M. J. WEBER. RAS and RAF-1 form signalling complex with MEK-1 but not MEK-2. *Mol. Cell. Biol.* 14: 8212–8218, 1994.
162. JIANG, Y., C. CHEN, Z. LI, W. GUO, G. A. GEGNER, S. LIN, AND J. HAN. Characterization of the structure and function of a new mitogen-activated protein kinase (p38β). *J. Biol. Chem.* 271: 17920–17926, 1996.
163. JIANG, Y., H. GRAM, M. ZHAO, L. NEW, J. GU, L. FENG, F. DI PADOVA, R. J. ULEVITCH, AND J. HAN. Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38δ. *J. Biol. Chem.* 272: 30122–30128, 1997.
164. JIMENEZ, L. A., C. ZANELLA, H. FUNG, Y. M. JANSSEN, P. VACEK, C. CHARLAND, J. GOLDBERG, AND B. T. MOSSMAN. Role of extracellular signal-regulated protein kinases in apoptosis by asbestos and H_2O_2. *Am. J. Physiol.* 273 (*Lung Cell. Mol. Physiol.* 17): L1029–L1035, 1997.
165. JOHNSON, L. N., M. E. NOBLE, AND D. J. OWEN. Active and inactive protein kinases: structural basis for regulation. *Cell* 85: 149–158, 1996.
166. JONAK, C., S. KIEGERL, W. LIGTERINK, P. J. BARKER, N. S. HUSKISSON, AND H. HIRT. Stress signaling in plants: a mitogen-activated protein kinase pathway is activated by cold and drought. *Proc. Natl. Acad. Sci. USA* 93: 11274–11279, 1996.
167. JONAK, C., S. KIEGERL, C. LLOYD, J. CHAN, AND H. HIRT. MMK2, a novel alfalfa MAP kinase, specifically complements the yeast MPK1 function. *Mol. Gen. Genet.* 248: 686–694, 1995.
168. JONAK, C., A. PAY, L. BOGRE, H. HIRT, AND E. HEBERLE-BORS. The plant homologue of a MAP kinase is expressed in a cell cycle-dependent and organ-specific manner. *Plant J.* 3: 611–617, 1993.
169. KALLUNKI, T., B. SU, I. TSIGELNY, H. K. SLUSS, B. DERIJARD, G. MOORE, R. J. DAVIS, AND M. KARIN. JNK2 contains a specificity-

determining region responsible for efficient c-Jun binding and phosphorylation. *Genes Dev.* 8: 2996–3007, 1994.
170. KASIBHATLA, S., T. BRUNNER, L. GENESTIER, F. ECHEVERRI, A. MAHBOUBI, AND D. R. GREEN. DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kB and AP-1. *Mol. Cell* 1: 543–551, 1998.
171. KATO, T. J., K. OKAZAKI, H. MURAKAMI, S. STETTLER, P. A. FANTES, AND H. OKAYAMA. Stress signal, mediated by a Hog1-like MAP kinase, controls sexual development in fission yeast. *FEBS Lett.* 378: 207–212, 1996.
172. KATO, Y., V. V. KRAVCHENKO, R. I. TAPPING, J. HAN, R. J. ULEVITCH, AND J. D. LEE. BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *EMBO J.* 16: 7054–7066, 1997.
173. KATOH, M., M. HIRAI, T. SUGIMURA, AND M. TERADA. Cloning and characterization of MST, a novel (putative) serine/threonine kinase with SH3 domain. *Oncogene* 10: 1447–1451, 1995.
174. KAZLAUSKAS, A., AND J. A. COOPER. Protein kinase C mediates platelet-derived growth factor-induced tyrosine phosphorylation of p42. *J. Cell Biol.* 106: 1395–1402, 1988.
175. KIEFER, F., L. A. TIBBLES, M. ANAFI, A. JANSSEN, B. W. ZANKE, N. LASSAM, T. PAWSON, J. R. WOODGETT, AND N. N. ISCOVE. HPK1, a hematopoietic protein kinase activating the SAPK/JNK pathway. *EMBO J.* 15: 7013–7025, 1996.
176. KNAUS, U. G., S. MORRIS, H. J. DONG, J. CHERNOFF, AND G. M. BOKOCH. Regulation of human leukocyte p21-activated kinases through G protein-coupled receptors. *Science* 269: 221–223, 1995.
177. KNIGHTON, D. R., J. H. ZHENG, L. F. TEN EYCK, V. A. ASHFORD, N. H. XUONG, S. S. TAYLOR, AND J. M. SOWADSKI. Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science* 253: 407–414, 1991.
178. KNIGHTON, D. R., J. H. ZHENG, L. F. TEN EYCK, N. H. XUONG, S. S. TAYLOR, AND J. M. SOWADSKI. Structure of a peptide inhibitor bound to the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science* 253: 414–420, 1991.
179. KOIDE, H., T. SATOH, M. NAKAFUKU, AND Y. KAZIRO. GTP-dependent association of Raf-1 with Ha-Ras: identification of Raf as a target downstream of Ras in mammalian cells. *Proc. Natl. Acad. Sci. USA* 90: 8683–8686, 1993.
180. KOLCH, W., G. HEIDECKER, P. LLOYD, AND U. R. RAPP. Raf-1 protein kinase is required for growth of induced NIH/3T3 cells. *Nature* 349: 426–428, 1991.
181. KORNFELD, K., K. L. GUAN, AND H. R. HORVITZ. The *Caenorhabditis elegans* gene mek-2 is required for vulval induction and encodes a protein similar to the protein kinase MEK. *Genes Dev.* 9: 756–768, 1995.
182. KOSAKO, H., E. NISHIDA, AND Y. GOTOH. cDNA cloning of MAP kinase kinase reveals kinase cascade pathways in yeasts to vertebrates. *EMBO J.* 12: 787–794, 1993.
183. KRAMER, R. M., E. F. ROBERTS, B. A. STRIFFLER, AND E. M. JOHNSTONE. Thrombin induces activation of p38 MAP kinase in human platelets. *J. Biol. Chem.* 270: 27395–27398, 1995.
184. KRANZ, J. E., B. SATTERBERG, AND E. A. ELION. The MAP kinase Fus3 associates with and phosphorylates the upstream signaling component Ste5. *Genes Dev.* 8: 313–327, 1994.
185. KRISAK, L., R. STRICH, R. S. WINTERS, J. P. HALL, M. J. MALLORY, D. KREITZER, R. S. TUAN, AND E. WINTER. SMK1, a developmentally regulated MAP kinase, is required for spore wall assembly in *Saccharomyces cerevisiae*. *Genes Dev.* 8: 2151–2161, 1994.
186. KRSTIC, M. D., I. ROGATSKY, K. R. YAMAMOTO, AND M. J. GARABEDIAN. Mitogen-activated and cyclin-dependent protein kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. *Mol. Cell. Biol.* 17: 3947–3954, 1997.
187. KYRIAKIS, J. M., H. APP, X.-F. ZHANG, P. BANERJEE, D. L. BRAUTIGAN, U. R. RAPP, AND J. AVRUCH. Raf-1 activate MAP kinase-kinase. *Nature* 358: 417–421, 1992.
188. KYRIAKIS, J. M., P. BANERJEE, E. NIKOLAKAKI, T. DAI, E. A. RUBIE, M. F. AHMAD, J. AVRUCH, AND J. R. WOODGETT. The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 369: 156–160, 1994.
189. LACKNER, M. R., K. KORNFELD, L. M. MILLER, H. R. HORVITZ, AND S. K. KIM. A MAP kinase homolog, mpk-1, is involved in ras-mediated induction of vulval cell fates in *Caenorhabditis elegans*. *Genes Dev.* 8: 160–173, 1994.
190. LANGE-CARTER, C. A., AND G. L. JOHNSON. Ras-dependent growth factor regulation of MEK kinase in PC12 cells. *Science* 265: 1458–1461, 1994.
191. LANGE-CARTER, C. A., C. M. PLEIMAN, A. M. GARDNER, K. J. BLUMER, AND G. L. JOHNSON. A divergence in the MAP kinase regulatory network defined by MEK kinase and Raf. *Science* 260: 315–319, 1993.
192. LASSIGNAL JOHNSON, N., A. M. GARDNER, K. M. DIENER, C. A. LANGE-CARTER, J. GLEAVY, M. B. JARPE, A. MINDEN, M. KARIN, L. I. ZON, AND G. L. JOHNSON. Signal transduction pathways regulated by mitogen-activated/extracellular response kinase kinase kinase induce cell death. *J. Biol. Chem.* 271: 3229–3237, 1996.
193. LAVOIE, J. N., G. L'ALLEMAIN, A. BRUNET, R. MÜLLER, AND J. POUYSSEGUR. Cyclin D1 expression is regulated positively by the $p42/44^{MAPK}$ and negatively by the $p38/HOG^{MAPK}$ pathway. *J. Biol. Chem.* 271: 20608–20616, 1996.
194. LAVOIE, J. N., N. RIVARD, G. L'ALLEMAIN, AND J. POUYSSEGUR. A temporal and biochemical link between growth factor-activated MAP kinases, cyclin D1 induction and cell cycle entry. *Prog. Cell Cycle Res.* 2: 49–58, 1996.
195. LECHNER, C., M. A. ZAHALKA, J. F. GIOT, N. P. MOLLER, AND A. ULLRICH. ERK6, a mitogen-activated protein kinase involved in C2C12 myoblast differentiation. *Proc. Natl. Acad. Sci. USA* 93: 4355–4359, 1996.
196. LEE, F. S., J. HAGLER, Z. J. CHEN, AND T. MANIATIS. Activation of the I-kappa-Bα kinase complex by MEKK1, a kinase of the JNK pathway. *Cell* 88: 213–222, 1997.
197. LEE, J. C., J. T. LAYDON, P. C. McDONNELL, T. F. GALLAGHER, S. KUMAR, D. GREEN, D. McNULTY, M. J. BLUMENTHAL, J. R. HEYS, S. W. LANDVATTER, J. E. STRICKLER, M. M. McLAUGHLIN, I. R. SIEMENS, S. M. FISHER, G. P. LIVI, J. R. WHITE, J. L. ADAMS, AND P. R. YOUG. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372: 739–746, 1994.
198. LEE, J. E., T. W. BECK, U. BRENNSCHEIDT, L. J. DEGENNARO, AND U. R. RAPP. The complete sequence and promoter activity of the human A-raf-1 gene (ARAF1). *Genomics* 20: 43–55, 1994.
199. LEE, K. S., K. IRIE, Y. GOTOH, Y. WATANABE, H. ARAKI, E. NISHIDA, K. MATSUMOTO, AND D. E. LEVIN. A yeast mitogen-activated protein kinase homolog (Mpk1p) mediates signalling by protein kinase C. *Mol. Cell. Biol.* 13: 3067–3075, 1993.
200. LEE, K. S., AND D. E. LEVIN. Dominant mutations in a gene encoding a putative protein kinase (BCK1) bypass the requirement for a *Saccharomyces cerevisiae* protein kinase C homolog. *Mol. Cell. Biol.* 12: 172–182, 1992.
201. LEEVERS, S. J., H. F. PATERSON, AND C. J. MARSHALL. Requirement for Ras in Raf activation is overcome by targetting Raf to the plasma membrane. *Nature* 369: 411–414, 1994.
202. LE GUELLEC, R., K. LE GUELLEC, J. PARIS, AND M. PHILIPPE. Nucleotide sequence of *Xenopus* C-raf coding region. *Nucleic Acids Res.* 16: 10357, 1988.
203. LENORMAND, P., C. SARDET, G. PAGES, G. L'ALLEMAIN, A. BRUNET, AND J. POUYSSEGUR. Growth factors induce nuclear translocation of MAP kinases (p42mapk and p44mapk) but not their activator MAP kinase kinase (p45mapkk) in fibroblasts. *J. Cell Biol.* 122: 1079–1088, 1993.
204. LEROSEY, I., V. PIZON, A. TAVITIAN, AND J. DE GUNZBURG. The cAMP-dependent protein kinase phosphorylates the rap1 protein in vitro as well as in intact fibroblasts, but not the closely related rap2 protein. *Biochem. Biophys. Res. Commun.* 175: 430–436, 1991.
205. LEV, S., H. MORENO, R. MARTINEZ, P. CANOLL, E. PELES, J. M. MUSACCHIO, G. D. PLOWMAN, B. RUDY, AND J. SCHLESSINGER. Protein tyrosine kinase PYK2 involved in Ca^{2+}-induced regulation of ion channel and MAP kinase functions. *Nature* 376: 737–745, 1995.
206. LEVIN, D. E., AND E. BARTLETT-HEUBUSCH. Mutants in the *S. cerevisiae* PKC1 gene display a cell cycle-specific osmotic stability defect. *J. Cell Biol.* 116: 1221–1229, 1992.
207. LI, D., L. ROGERS, AND P. E. KOLATTUKUDY. Cloning and expres-

sion of cDNA encoding a mitogen-activated protein kinase from a phytopathogenic filamentous fungus. *Gene* 195: 161–166, 1997.
208. LIGTERINK, W., T. KROJ, U. ZUR NIEDEN, H. HIRT, AND D. SCHEEL. Receptor-mediated activation of a MAP kinase in pathogen defense of plants. *Science* 276: 2054–2057, 1997.
209. LIM, R., AND A. ZAHEER. In vitro enhancement of p38 mitogen-activated protein kinase activity by phosphorylated glia maturation factor. *J. Biol. Chem.* 271: 22953–22956, 1996.
210. LIN, L.-L., M. WARTMANN, A. Y. LIN, J. L. KNOPF, A. SETH, AND R. J. DAVIS. $cPLA_2$ is phosphorylated and activated by MAP kinase. *Cell* 72: 269–278, 1993.
211. LIU, Z.-G., H. HSU, D. V. GOEDDEL, AND M. KARIN. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kB activation prevents cell death. *Cell* 87: 565–576, 1996.
212. LOHIA, A., AND J. SAMUELSON. Molecular cloning of an *Entamoeba histolytica* gene encoding a putative mos family serine/threonine-kinase. *Biochim. Biophys. Acta* 1222: 122–124, 1994.
213. LOPEZ-ILASACA, M., P. CRESPO, P. G. PELLICI, J. S. GUTKIND, AND R. WETZKER. Linkage of G protein-coupled receptors to the MAPK signaling pathway through PI 3-kinase gamma. *Science* 275: 394–397, 1997.
214. LU, W., S. KATZ, R. GUPTA, AND B. J. MAYER. Activation of Pak by membrane localization mediated by an SH3 domain from the adaptor protein Nck. *Curr. Biol.* 7: 85–94, 1997.
215. LUTTRELL, L. M., G. J. DELLA ROCCA, T. VAN BIESEN, D. K. LUTTRELL, AND R. J. LEFKOWITZ. $G\beta\gamma$ subunits mediate Src-dependent phosphorylation of the epidermal growth factor receptor. A scaffold for G protein-coupled receptor-mediated Ras activation. *J. Biol. Chem.* 272: 4637–4644, 1997.
216. MA, H., M. GAMPER, C. PARENT, AND R. A. FIRTEL. The *Dictyostelium* MAP kinase kinase DdMEK1 regulates chemotaxis and is essential for chemoattractant-mediated activation of guanylyl cyclase. *EMBO J.* 16: 4317–4332, 1997.
217. MADHANI, H. D., C. A. STYLES, AND G. R. FINK. MAP kinases with distinct inhibitory functions impart signaling specificity during yeast differentiation. *Cell* 91: 673–684, 1997.
218. MAEDA, M., L. AUBRY, R. INSALL, C. GASKINS, P. N. DEVREOTES, AND R. A. FIRTEL. Seven helix chemoattractant receptors transiently stimulate mitogen-activated protein kinase in *Dictyostelium*. Role of heterotrimeric G proteins. *J. Biol. Chem.* 271: 3351–3354, 1996.
219. MAEDA, M., AND R. A. FIRTEL. Activation of the mitogen-activated protein kinase ERK2 by the chemoattractant folic acid in *Dictyostelium*. *J. Biol. Chem.* 272: 23690–23695, 1997.
220. MAEDA, T., M. TAKEKAWA, AND H. SAITO. Activation of yeast PBS2 MAPKK by MAPKKKs or by binding of an SH_3-containing osmosensor. *Science* 269: 554–558, 1995.
221. MANSER, E., T. LEUNG, H. SALIHUDDIN, Z. S. ZHAO, AND L. LIM. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature* 367: 40–46, 1994.
222. MARAIS, R., Y. LIGHT, C. MASON, H. PATERSON, M. F. OLSON, AND C. J. MARSHALL. Requirement of Ras-GTP-Raf complexes for activation of Raf-1 by protein kinase C. *Science* 280: 109–112, 1998.
223. MARAIS, R., Y. LIGHT, H. F. PATERSON, AND C. J. MARSHALL. Ras recruits Raf-1 to the plasma membrane for activation by tyrosine phosphorylation. *EMBO J.* 14: 3136–3145, 1995.
224. MARAIS, R., Y. LIGHT, H. F. PATERSON, C. S. MASON, AND C. J. MARSHALL. Differential regulation of Raf-1, A-Raf, and B-Raf by oncogenic Ras and tyrosine kinases. *J. Biol. Chem.* 272: 4378–4383, 1997.
225. MARAIS, R., AND C. J. MARSHALL. Control of the ERK MAP kinase cascade by Ras and Raf. *Cancer Surv.* 27: 101–125, 1996.
226. MARCUS, S., A. POLVERINO, M. BARR, AND M. WIGLER. Complexes between STE5 and components of the pheromone-responsive mitogen-activated protein kinase module. *Proc. Natl. Acad. Sci. USA* 91: 7762–7766, 1994.
227. MARQUARDT, B., D. FRITH, AND S. STABEL. Signalling from TPA to MAP kinase requires protein kinase C, raf and MEK: reconstitution of the signalling pathway in vitro. *Oncogene* 9: 3213–3218, 1994.
228. MARTIN, J. H., A. A. MOHIT, AND C. A. MILLER. Developmental expression in the mouse nervous system of the p493F12 SAP kinase. *Brain Res.* 35: 47–57, 1996.
229. MAYO, M. W., C.-Y. WANG, P. C. COGSWELL, K. S. ROGERS-GRAHAM, S. W. LOWE, C. J. DER, AND A. S. J. BALDWIN. Requirement of NF-kB activation to suppress p53-independent apoptosis induced by oncogenic Ras. *Science* 278: 1812–1815, 1997.
230. McTIGUE, M. A., S. L. BERNSTEIN, D. R. WILLIAMS, AND J. A. TAINER. Purification and crystallization of a schistosomal glutathione *S*-transferase. *Proteins* 22: 55–57, 1995.
231. MELOCHE, S., B. G. BEATTY, AND J. PELLERIN. Primary structure, expression and chromosomal locus of a human homolog of rat ERK3. *Oncogene* 13: 1575–1579, 1996.
232. MERTENS, S., M. CRAXTON, AND M. GOEDERT. SAP kinase-3, a new member of the family of mammalian stress-activated protein kinases. *FEBS Lett.* 383: 273–276, 1996.
233. MEYER, C. F., X. WANG, C. CHANG, D. TEMPLETON, AND T.-H. TAN. Interaction between c-Rel and the mitogen-activated protein kinase kinase kinase 1 signaling cascade in mediating kB enhancer activation. *J. Biol. Chem.* 271: 8971–8976, 1996.
234. MICHAUD, N. R., J. R. FABIAN, K. D. MATHES, AND D. K. MORRISON. 14-3-3 is not essential for Raf-1 function: identification of Raf-1 proteins that are biologically activated in a 14-3-3- and Ras-independent manner. *Mol. Cell. Biol.* 15: 3390–3397, 1995.
235. MIKI, T., T. P. FLEMING, M. CRESCENZI, C. J. MOLLOY, S. B. BLAM, S. H. REYNOLDS, AND S. A. AARONSON. Development of a highly efficient expression cDNA cloning system: application to oncogene isolation. *Proc. Natl. Acad. Sci. USA* 88: 5167–5171, 1991.
236. MILLAR, J. B., P. RUSSELL, J. E. DIXON, AND K. L. GUAN. Negative regulation of mitosis by two functionally overlapping PTPases in fission yeast. *EMBO J.* 11: 4943–4952, 1992.
237. MILNE, D. M., L. E. CAMPBELL, D. G. CAMPBELL, AND D. W. MEEK. p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation-induced protein kinase characteristic of the c-Jun kinase, JNK1. *J. Biol. Chem.* 270: 5511–5518, 1995.
238. MILTENBERGER, R. J., J. CORTNER, AND P. J. FARNHAM. An inhibitory Raf-1 mutant suppresses expression of a subset of v-*raf*-activated genes. *J. Biol. Chem.* 268: 15674–15680, 1993.
239. MINSHULL, J., H. SUN, N. K. TONKS, AND A. W. MURRAY. A MAP kinase-dependent spindle assembly checkpoint in *Xenopus* egg extracts. *Cell* 79: 475–486, 1994.
240. MISCHAK, H., T. SEITZ, P. JANOSCH, M. EULITZ, H. STEEN, M. SCHELLERER, A. PHILIPP, AND W. KOLCH. Negative regulation of Raf1 by phosphorylation of serine 621. *Mol. Cell. Biol.* 16: 5409–5418, 1996.
241. MITCHELL, A. P. Control of meiotic gene expression in *Saccharomyces cerevisiae*. *Microbiol. Rev.* 58: 56–70, 1994.
242. MIYAMOTO, S., S. TERAMOTO, O. A. COSO, J. S. GUTKIND, P. D. BURBELO, S. K. AKIYAMA, AND K. M. YAMADA. Integrin function: molecular hierarchies of cytoskeletal and signaling molecules. *J. Cell Biol.* 131: 791–805, 1995.
243. MIYOSHI, J., T. HIGASHI, H. MUKAI, T. OHUCHI, AND T. KAKUNAGA. Structure and transforming potential of the human *cot* oncogene encoding a putative protein kinase. *Mol. Cell. Biol.* 11: 4088–4096, 1991.
244. MIZOGUCHI, T., Y. GOTOH, E. NISHIDA, K. YAMAGUCHI-SHINOZAKI, N. HAYASHIDA, T. IWASAKI, H. KAMADA, AND K. SHINOZAKI. Characterization of two cDNAs that encode MAP kinase homologues in *Arabidopsis thaliana* and analysis of the possible role of auxin in activating such kinase activities in cultured cells. *Plant J.* 5: 111–122, 1994.
245. MIZOGUCHI, T., N. HAYASHIDA, K. YAMAGUCHI-SHINOZAKI, H. KAMADA, AND K. SHINOZAKI. ATMPKs: a gene family of plant MAP kinases in *Arabidopsis thaliana*. *FEBS Lett.* 336: 440–444, 1993.
246. MIZOGUCHI, T., K. IRIE, T. HIRAYAMA, N. HAYASHIDA, K. YAMAGUCHI-SHINOZAKI, K. MATSUMOTO, AND K. SHINOZAKI. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, water stress in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 93: 765–769, 1996.
247. MORIGUCHI, T., N. KUROYANAGI, K. YAMAGUCHI, Y. GOTOH, K. IRIE, T. KANO, K. SHIRAKABE, Y. MURO, H. SHIBUYA, K. MATSUMOTO, E. NISHIDA, AND M. HAGIWARA. A novel kinase cascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. *J. Biol. Chem.* 271: 13675–13679, 1996.

248. MORIGUCHI, T., F. TOYOSHIMA, Y. GOTOH, A. IWAMATSU, K. IRIE, E. MORI, N. KUROYANAGI, M. HAGIWARA, K. MATSUMOTO, AND E. NISHIDA. Purification and identification of a major activator for p38 from osmotically shocked cells. Activation of mitogen-activated protein kinase kinase 6 by osmotic shock, tumor necrosis factor-alpha, and H_2O_2. *J. Biol. Chem.* 271: 26981–26988, 1996.
249. MORINO, N., T. MIMURA, K. HAMASAKI, K. TOBE, K. UEKI, K. KIKUCHI, K. TAKEHARA, T. KADOWAKI, Y. YAZAKI, AND Y. NOJIMA. Matrix/integrin interaction activates the mitogen-activated protein kinase, $p44^{erk-1}$ and $p42^{erk-2}$. *J. Biol. Chem.* 270: 269–273, 1995.
250. MOSCH, H. U., R. L. ROBERTS, AND G. R. FINK. Ras2 signals via the Cdc42/Ste20/mitogen-activated protein kinase module to induce filamentous growth in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 93: 5352–5356, 1996.
251. MUDA, M., U. BOSCHERT, R. DICKINSON, J.-C. MARTINOU, I. MARTINOU, M. CAMPS, W. SCHLEGEL, AND S. ARKINSTALL. MKP-3, a novel cytosolic protein-tyrosine phosphatase that exemplifies a new class of mitogen-activated protein kinase phosphatase. *J. Biol. Chem.* 271: 4319–4326, 1996.
252. MUDA, M., U. BOSCHERT, A. SMITH, B. ANTONSSON, C. GILLIERON, C. CHABERT, M. CAMPS, I. MARTINOU, A. ASHWORTH, AND S. ARKINSTALL. Molecular cloning and functional characterization of a novel mitogen-activated protein kinase phosphatase, MKP-4. *J. Cell Biol.* 272: 5141–5151, 1997.
253. MUDA, M., A. THEODOSIOU, N. RODRIGUES, U. BOSCHERT, M. CAMPS, C. GILLIERON, K. DAVIES, A. ASHWORTH, AND S. ARKINSTALL. The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. *J. Biol. Chem.* 271: 27205–27208, 1996.
254. MUSLIN, A. J., A. M. MACNICOL, AND L. T. WILLIAMS. Raf-1 protein kinase is important for progesterone-induced *Xenopus* oocyte maturation and acts downstream of mos. *Mol. Cell. Biol.* 13: 4197–4202, 1993.
255. MUSLIN, A. J., J. W. TANNER, P. M. ALLEN, AND A. S. SHAW. Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine. *Cell* 84: 889–897, 1996.
256. MUSTI, A. M., M. TREIER, AND D. BOHMANN. Reduced ubiquitin-dependent degradation of c-Jun after phosphorylation by MAP kinases. *Science* 275: 400–402, 1997.
257. NADIN-DAVIS, S. A., AND A. NASIM. A gene which encodes a predicted protein kinase can restore some functions of the ras gene in fission yeast. *EMBO J.* 7: 985–993, 1988.
258. NAVARRO-GARCIA, F., M. SANCHEZ, J. PLA, AND C. NOMBELA. Functional characterization of the MCK1 gene of *Candida albicans*, which encodes a mitogen-activated protein kinase homolog related to cell integrity. *Mol. Cell. Biol.* 15: 2197–2206, 1995.
259. NEIMAN, A. M. Conservation and reiteration of a kinase cascade. *Trends Genet.* 9: 390–394, 1993.
260. NEIMAN, A. M., B. J. STEVENSON, H. P. XU, G. F. SPRAGUE, I. HERSKOWITZ, M. WIGLER, AND S. MARCUS. Functional homology of protein kinases required for sexual differentiation in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* suggests a conserved signal transduction module in eukaryotic organisms. *Mol. Biol. Cell* 4: 107–120, 1993.
261. NGUYEN, T. T., J. C. SCIMECA, C. FILLOUX, P. PERALDI, J. L. CARPENTIER, AND E. VAN OBBERGHEN. Co-regulation of the mitogen-activated protein kinase, extracellular signal-regulated kinase 1, and the 90-kDa ribosomal S6 kinase in PC12 cells. Distinct effects of the neurotrophic factor, nerve growth factor, and the mitogenic factor, epidermal growth factor. *J. Biol. Chem.* 268: 9803–9810, 1993.
262. NIELSEN, O., J. DAVEY, AND R. EGEL. The ras1 function of *Schizosaccharomyces pombe* mediates pheromone-induced transcription. *EMBO J.* 11: 1391–1395, 1992.
263. NISHIDA, Y., M. HATA, T. AYAKI, H. RYO, M. YAMAGATA, K. SHIMIZU, AND Y. NISHIZUKA. Proliferation of both somatic and germ cells is affected in the *Drosophila* mutants of raf proto-oncogene. *EMBO J.* 7: 775–781, 1988.
264. NISHIHAMA, R., H. BANNO, E. KAWAHARA, K. IRIE, AND Y. MACHIDA. Possible involvement of differential splicing in regulation of the activity of *Arabidopsis* ANP1 that is related to mitogen-activated protein kinase kinase kinases (MAPKKKS). *Plant J.* 12: 39–48, 1997.
265. NISHINA, H., K. D. FISCHER, L. RADVANYI, A. SHAHINIAN, R. HAKEM, E. A. RUBIE, A. BERNSTEIN, T. W. MAK, J. R. WOODGETT, AND J. M. PENNINGER. Stress-signalling kinase Sek1 protects thymocytes from apoptosis mediated by CD95 and CD3. *Nature* 385: 350–353, 1997.
266. OEHLEN, L. J., J. D. MCKINNEY, AND F. R. CROSS. Ste12 and Mcm1 regulate cell cycle-dependent transcription of FAR1. *Mol. Cell. Biol.* 16: 2830–2837, 1996.
267. OGATA, A., D. CHAUHAN, G. TEOH, S. P. TREON, M. URASHIMA, R. L. SCHLOSSMAN, AND K. C. ANDERSON. IL-6 triggers cell growth via the Ras-dependent mitogen-activated protein kinase cascade. *J. Immunol.* 159: 2212–2221, 1997.
268. OHARA, R., J. MIYOSHI, M. AOKI, AND K. TOYOSHIMA. The murine cot proto-oncogene: genome structure and tissue-specific expression. *Jpn. J. Cancer Res.* 84: 518–525, 1993.
269. OTSU, M., Y. TERADA, AND H. OKAYAMA. Isolation of two members of the rat MAP kinase kinase gene family. *FEBS Lett.* 320: 246–250, 1993.
270. OWAKI, H., R. MAKAR, T. G. BOULTON, M. H. COBB, AND T. D. GEPPERT. Extracellular signal-regulated kinases in T cells: characterization of human ERK1 and ERK2 cDNAs. *Biochem. Biophys. Res. Commun.* 182: 1416–1422, 1992.
271. PAGES, G., A. BRUNET, G. L'ALLEMAIN, AND J. POUYSSEGUR. Constitutive mutant and putative regulatory serine phosphorylation site of mammalian MAP kinase kinase (MEK1). *EMBO J.* 13: 3003–3010, 1994.
272. PAGES, G., P. LENORMAND, G. L'ALLEMAIN, J. C. CHAMBARD, S. MELOCHE, AND J. POUYSSEGUR. Mitogen-activated protein kinase p42mapk and p44mapk are required for fibroblast proliferation. *Proc. Natl. Acad. Sci. USA* 90: 8319–8323, 1993.
273. PATRIOTIS, C., A. MAKRIS, S. E. BEAR, AND P. N. TSICHLIS. Tumor progression locus 2 (Tpl-2) encodes a protein kinase involved in the progression of rodent T-cell lymphomas and in T-cell activation. *Proc. Natl. Acad. Sci. USA* 90: 2251–2255, 1993.
274. PENG, X., J. M. ANGELASTRO, AND L. A. GREENE. Tyrosine phosphorylation of extracellular signal-regulated protein kinase 4 in response to growth factors. *J. Neurochem.* 66: 1191–1197, 1996.
275. PETER, M., A. GARTNER, J. HORECKA, G. AMMERER, AND I. HERSKOWITZ. FAR1 links the signal transduction pathway to the cell cycle machinery in yeast. *Cell* 73: 747–760, 1993.
276. POSADA, J., N. YEW, N. G. AHN, G. F. VANDE WOUDE, AND J. A. COOPER. Mos stimulates MAP kinase in *Xenopus* oocytes and activates a MAP kinase kinase in vitro. *Mol. Cell. Biol.* 13: 2546–2553, 1993.
277. POSAS, F., AND H. SAITO. Osmotic activation of the hog MAPK pathway via Ste11p MAPKKK: scaffold role of Pbs2p MAPKK. *Science* 276: 1702–1705, 1997.
278. POSAS, F., S. M. WURGLER-MURPHY, T. MAEDA, E. A. WITTEN, T. C. THAI, AND H. SAITO. Yeast HOG1 MAP kinase cascade is regulated by a multistep phosphorelay mechanism in the Sln1-Ypd1-Ssk1 "two component" osmosensor. *Cell* 86: 865–875, 1996.
279. POTAPOVA, O., A. HAGHIGHI, F. BOST, C. LIU, M. J. BIRRER, R. GJERSET, AND D. MERCOLA. The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. *J. Biol. Chem.* 272: 14041–14044, 1997.
280. PRINTEN, J. A., AND G. F. J. SPRAGUE. Protein-protein interaction in the yeast pheromone response pathway: Ste5p interacts with all members of the MAP kinase cascade. *Genetics* 138: 609–619, 1994.
281. PULVERER, B. J., J. M. KYRIAKIS, J. AVRUCH, E. NIKOLAKAKI, AND J. R. WOODGETT. Phosphorylation of c-Jun mediated by MAP kinases. *Nature* 354: 494–496, 1991.
282. QUI, M. S., AND S. H. GREEN. PC12 cell neuronal differentiation is associated with prolonged p21ras activity and consequent prolonged ERK activity. *Neuron* 9: 705–717, 1992.
283. RACKE, F. K., K. LEWANDOSWSKA, S. GOUELI, AND A. N. GOLDFARB. Sustained activation of the extracellular signal-regulated kinase/mitogen-activated protein kinase pathway is required for megakaryocytic differentiation of K562 cells. *J. Biol. Chem.* 272: 23366–23370, 1997.
284. RAINGEAUD, J., S. GUPTA, J. S. ROGERS, M. DICKENS, J. HAN, R. J. ULEVITCH, AND R. J. DAVIS. Pro-inflammatory cytokines and

environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J. Biol. Chem.* 270: 7420–7426, 1995.
285. RAINGEAUD, J., A. J. WHITMARSH, T. BARRETT, B. DERIJARD, AND R. J. DAVIS. MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Mol. Cell. Biol.* 16: 1247–1255, 1996.
286. RANA, A., K. GALLO, P. GODOWSKI, S.-I. HIRAI, S. OHNO, L. ZON, J. M. KYRIAKIS, AND J. AVRUCH. The mixed lineage kinase SPRK phosphorylates and activates the stress-activated protein kinase activator, SEK-1. *J. Biol. Chem.* 271: 19025–19028, 1996.
287. RAY, L. B., AND T. W. STURGILL. Insulin-stimulated microtubule-associated protein kinase is phosphorylated on tyrosine and threonine in vivo. *Proc. Natl. Acad. Sci. USA* 85: 3753–3757, 1988.
288. REDDY, U. R., AND D. PLEASURE. Cloning of a novel putative protein kinase having a leucine zipper domain from human brain. *Biochem. Biophys. Res. Commun.* 202: 613–620, 1994.
289. REDDY, U. R., AND D. PLEASURE. Cloning of a novel putative protein kinase having a leucine zipper domain from human brain (Erratum). *Biochem. Biophys. Res. Commun.* 205: 1494–1495, 1994.
290. RENSHAW, M. W., D. TOKSOZ, AND M. A. SCHWARTZ. Involvement of the small GTPase Rho in integrin-mediated activation of mitogen-activated protein kinase. *J. Biol. Chem.* 271: 21691–21694, 1996.
291. REUTHER, G. W., AND A. M. PENDERGAST. The roles of 14-3-3 proteins in signal transduction. *Vitam. Horm.* 52: 149–175, 1996.
292. RIESGO-ESCOVAR, J. R., M. JENNI, A. FRITZ, AND E. HAFEN. The *Drosophila* Jun-N-terminal kinase is required for cell morphogenesis but not for DJun-dependent cell fate specification in the eye. *Genes Dev.* 10: 2759–2768, 1996.
293. ROBERTS, R. L., AND G. R. FINK. Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. *Genes Dev.* 8: 2974–2985, 1994.
294. ROSSOMANDO, A. J., D. M. PAYNE, M. J. WEBER, AND T. W. STURGILL. Evidence that pp42, a major tyrosine kinase target protein, is a mitogen-activated serine/threonine protein kinase. *Proc. Natl. Acad. Sci. USA* 86: 6940–6943, 1989.
295. ROUSE, J., P. COHEN, S. TRIGON, M. MORANGE, A. ALONSO-LLAMAZARES, D. ZAMANILLO, T. HUNT, AND A. R. NEBREDA. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell* 78: 1027–1037, 1994.
296. RUSSELL, M., C. A. LANGE-CARTER, AND G. L. JOHNSON. Direct interaction between Ras and the kinase domain of mitogen-activated protein kinase kinase kinase (MEKK1). *J. Biol. Chem.* 270: 11757–11760, 1995.
297. SAGATA, N., M. OSKARSSON, T. COPELAND, J. BRUMBAUGH, AND G. F. VANDE WOUDE. Function of c-*mos* proto-oncogene product in meiotic maturation in *Xenopus* oocytes. *Nature* 335: 519–525, 1988.
298. SALMERON, A., T. B. AHMAD, G. W. CARLILE, D. PAPPIN, R. P. NARSIMHAN, AND S. C. LEY. Activation of MEK-1 and SEK-1 by Tpl-2 proto-oncoprotein, a novel MAP kinase kinase kinase. *EMBO J.* 15: 817–826, 1996.
299. SAMEJIMA, I., S. MACKIE, AND P. A. FANTES. Multiple modes of activation of the stress-responsive MAP kinase pathway in fission yeast. *EMBO J.* 16: 6162–6170, 1997.
300. SANCHEZ, I., R. T. HUGHES, B. J. MAYER, K. YEE, J. R. WOODGETT, J. AVRUCH, J. M. KYRIAKIS, AND L. I. ZON. Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun. *Nature* 372: 794–798, 1994.
301. SASAOKA, T., W. J. LANGLOIS, J. W. LEITNER, B. DRAZNIN, AND J. M. OLEFSKY. The signaling pathway coupling epidermal growth factor receptors to activation of p21ras. *J. Biol. Chem.* 269: 32621–32625, 1994.
302. SAUMA, S., AND E. FRIEDMAN. Increased expression of protein kinase C beta activates ERK3. *J. Biol. Chem.* 271: 11422–11426, 1996.
304. SCHMIDT, M., M. K. OSKARSSON, J. K. DUNN, D. G. BLAIR, S. HUGHES, F. PROPST, AND G. F. VANDE WOUDE. Chicken homolog of the mos proto-oncogene. *Mol. Cell. Biol.* 8: 923–929, 1988.
305. SCHULTZ, J., B. FERGUSSON, AND G. F. SPRAGUE. Signal transduction and growth control in yeast. *Curr. Biol.* 5: 31–37, 1995.
306. SCHWENGER, P., P. BELLOSTA, I. VIETOR, C. BASILICO, E. Y. SKOLNIK, AND J. VILCEK. Sodium salicylate induces apoptosis via p38 mitogen activated protein kinase but inhibits tumor necrosis factor-induced c-Jun N-terminal kinase/stress-activated protein kinase activation. *Proc. Natl. Acad. Sci. USA* 94: 2869–2873, 1997.
307. SEGALL, J. E., A. KUSPA, G. SHAULSKY, M. ECKE, M. MAEDA, C. GASKINS, R. A. FIRTEL, AND W. F. LOOMIS. A MAP kinase necessary for receptor-mediated activation of adenylyl cyclase in *Dictyostelium*. *J. Cell Biol.* 128: 405–413, 1995.
308. SEGER, R., AND E. G. KREBS. The MAPK signaling cascade. *FASEB J.* 9: 726–735, 1995.
309. SEGER, R., D. SEGER, F. J. LOZEMAN, N. G. AHN, L. M. GRAVES, J. S. CAMPBELL, L. ERICSSON, M. HARRYLOCK, A. M. JENSEN, AND E. G. KREBS. Human T-cell mitogen-activated protein kinase kinases are related to yeast signal transduction kinases. *J. Biol. Chem.* 267: 25628–25631, 1992.
310. SEO, S., M. OKAMOTO, H. SETO, K. ISHIZUKA, H. SANO, AND Y. OHASHI. Tobacco MAP kinase: a possible mediator in wound signal transduction pathways. *Science* 270: 1988–1992, 1995.
311. SHIBATA, W., H. BANNO, Y. ITO, K. HIRANO, K. IRIE, S. USAMI, C. MACHIDA, AND Y. MACHIDA. A tobacco protein kinase, NPK2, has a domain homologous to a domain found in activators of mitogen-activated protein kinases (MAPKKs). *Mol. Gen. Genet.* 246: 401–410, 1995.
312. SHIEH, J. C., M. G. WILKINSON, V. BUCK, B. A. MORGAN, K. MAKINO, AND J. B. MILLAR. The Mcs4 response regulator coordinately controls the stress-activated Wak1-Wis1-Sty1 MAP kinase pathway and fission yeast cell cycle. *Genes Dev.* 11: 1008–1022, 1997.
313. SHIOAZAKI, K., M. SHIOZAKI, AND P. RUSSELL. Mcs4 mitotic catastrophe suppressor regulates the fission yeast cell cycle through the Wik1-Wis-1-Spc1 kinase cascade. *Mol. Biol. Cell* 8: 409–419, 1997.
314. SINGH, P., S. GHOSH, AND A. DATTA. A novel MAP-kinase kinase from *Candida albicans*. *Gene* 190: 99–104, 1997.
315. SIOW, Y. L., G. B. KALMAR, J. S. SANGHERA, T. TAI, S. S. OH, AND S. L. PELECH. Identification of two essential phosphorylated threonine residues in the catalytic domain of Mekk1. Indirect activation by Pak3 and protein kinase C. *J. Biol. Chem.* 272: 7586–7594, 1997.
316. SITHANANDAM, G., W. KOLCH, F. M. DUH, AND U. R. RAPP. Complete coding sequence of a human B-raf cDNA and detection of B-raf protein kinase with isozyme specific antibodies. *Oncogene* 5: 1775–1780, 1990.
317. SLUSS, H. K., Z. HAN, T. BARRETT, R. J. DAVIS, AND Y. T. IP. A JNK signal transduction pathway that mediates morphogenesis and immune response in *Drosophila*. *Genes Dev.* 10: 2745–2758, 1996.
318. SMITH, A., F. RAMOS-MORALES, A. ASHWORTH, AND M. COLLINS. A role for JNK/SAPK in proliferation, but not apoptosis, of IL-3-dependent cells. *Curr. Biol.* 7: 893–896, 1997.
319. SMITH, M. R., S. J. DEGUDICIBUS, AND D. W. STACEY. Requirement for c-*ras* proteins during viral oncogene transformation. *Nature* 320: 540–543, 1986.
320. STAFSTROM, J. P., M. ALTSCHULER, AND D. H. ANDERSON. Molecular cloning and expression of a MAP kinase homologue from pea. *Plant Mol. Biol.* 22: 83–90, 1993.
321. STANCATO, L. F., M. SAKATSUME, M. DAVIS, P. DENT, F. DONG, E. F. PETRICOIN, J. J. KROLEWSKI, O. SILBENNOINEN, P. SAHARINEN, J. PIERCE, C. J. MARSHALL, T. STURGILL, D. S. FINBLOOM, AND A. C. LARNER. Beta interferon and oncostatin M activate Raf-1 and mitogen-activated protein kinase through a JAK1-dependent pathway. *Mol. Cell. Biol.* 17: 3833–3840, 1997.
322. STEIN, B., H. BRADY, M. X. YANG, D. B. YOUNG, AND M. S. BARBOSA. Cloning and characterization of MEK6, a novel member of the mitogen-activated protein kinase kinase cascade. *J. Biol. Chem.* 271: 11427–11433, 1996.
323. STEIN, B., M. X. YANG, D. B. YOUNG, R. JANKNECHT, T. HUNTER, B. W. MURRAY, AND M. S. BARBOSA. p38-2, a novel mitogen-activated protein kinase with distinct properties. *J. Biol. Chem.* 272: 19509–19517, 1997.
324. STORM, S. M., J. L. CLEVELAND, AND U. R. RAPP. Expression of raf family proto-oncogenes in normal mouse tissues. *Oncogene* 5: 345–351, 1990.

325. SU, B., E. JACINTO, M. HIBI, T. KALLUNKI, M. KARIN, AND Y. BEN-NERIAH. JNK is involved in signal integration during costimulation of T lymphocytes. *Cell* 77: 727–736, 1994.
326. SU, Y.-C., J. HAN, S. XU, M. COBB, AND E. Y. SKOLNIK. NIK is a new Ste20-related kinase that binds NCK and MEKK1 and activates the SAPK/JNK cascade via a conserved regulatory domain. *EMBO J.* 16: 1279–1290, 1997.
327. SUGDEN, P. H., AND A. CLERK. Regulation of the ERK subgroup of MAP kinase cascades through G protein-coupled receptors. *Cell. Signal.* 9: 337–351, 1997.
328. SUNDARAM, M., AND M. HAN. The *C. elegans ksr-1* gene encodes a novel Raf-related kinase involved in Ras-mediated signal transduction. *Cell* 83: 889–901, 1995.
329. SUTHERLAND, C. L., A. W. HEATH, S. L. HEATH, P. R. YOUNG, AND M. R. GOLD. Differential activation of the ERK, JNK, and p38 mitogen-activated protein kinases by CD40 and the B cell antigen receptor. *J. Immunol.* 157: 3381–3390, 1996.
330. TAKEKAWA, M., F. POSAS, AND H. SAITO. A human homolog of the yeast Ssk2/Ssk22 MAP kinase kinase kinases, MTK1, mediates stress-induced activation of the p38 and JNK pathways. *EMBO J.* 16: 4973–4982, 1997.
331. TAKENAKA, K., Y. GOTOH, AND E. NISHIDA. MAP kinase is required for the spindle assembly checkpoint but is dispensable for the normal M phase entry and exit in *Xenopus* egg cell cycle extracts. *J. Cell Biol.* 136: 1091–1097, 1997.
332. TAMEMOTO, H., T. KADOWAKI, K. TOBE, K. UEKI, T. IZUMI, Y. CHATANI, M. KOHNO, M. KASUGA, Y. YAZAKI, AND Y. AKANUMA. Biphasic activation of two mitogen-activated protein kinases during the cell cycle in mammalian cells. *J. Biol. Chem.* 267: 20293–20297, 1992.
333. TANIGUCHI, T. Cytokine signaling through nonreceptor protein tyrosine kinases. *Science* 268: 251–255, 1995.
334. THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673–4680, 1994.
335. TIBBLES, L. A., Y. L. ING, F. KIEFER, J. CHAN, N. ISCOVE, J. R. WOODGETT, AND N. J. LASSAM. MLK-3 activates the SAPK/JNK and p38/RK pathways via SEK1 and MKK3/6. *EMBO J.* 15: 7026–7035, 1996.
336. TOBIUME, K., T. INAGE, K. TAKEDA, S. ENOMOTO, K. MIYAZONO, AND H. ICHIJO. Molecular cloning and characterization of the mouse apoptosis signal-regulating kinase 1. *Biochem. Biophys. Res. Commun.* 239: 905–910, 1997.
337. TODA, T., S. DHUT, G. SUPERTI-FURGA, Y. GOTOH, E. NISHIDA, R. SUGIURA, AND T. KUNO. The fission yeast *pmk1+* gene encodes a novel mitogen-activated protein kinase homolog which regulates cell integrity and functions coordinately with the protein kinase C pathway. *Mol. Cell. Biol.* 16: 6752–6764, 1996.
338. TODA, T., M. SHIMANUKI, AND M. YANAGIDA. Fission yeast genes that confer resistance to staurosporine encode an AP-1-like transcription factor and a protein kinase related to the mammalian ERK1/MAP2 and budding yeast FUS3 and KSS1 kinases. *Genes Dev.* 5: 60–73, 1991.
339. TORRES, L., H. MARTIN, M. I. GARCIA-SAEZ, J. ARROYO, M. MOLINA, M. SANCHEZ, AND C. NOMBELA. A protein kinase gene complements the lytic phenotype of *Saccharomyces cerevisiae* lyt2 mutants. *Mol. Microbiol.* 5: 2845–2854, 1991.
340. TOURNIER, C., A. J. WHITMARSH, J. CAVANAGH, T. BARRETT, AND R. J. DAVIS. Mitogen-activated protein kinase kinase 7 is an activator of the c-Jun NH_2-terminal kinase. *Proc. Natl. Acad. Sci. USA* 94: 7337–7342, 1997.
341. TSUDA, L., Y. H. INOUE, M. A. YOO, M. MIZUNO, M. HATA, Y. M. LIM, T. ADACHI-YAMADA, H. RYO, Y. MASAMUNE, AND Y. NISHIDA. A protein kinase similar to MAP kinase activator acts downstream of the raf kinase in *Drosophila*. *Cell* 72: 407–414, 1993.
342. UMBHAUER, M., C. J. MARSHALL, C. S. MASON, R. W. OLD, AND J. C. SMITH. Mesoderm induction in *Xenopus* caused by activation of MAP kinase. *Nature* 376: 58–62, 1995.
343. VAILLANCOURT, R. R., A. M. GARDNER, AND G. L. JOHNSON. B-Raf-dependent regulation of the MEK-1/mitogen-activated protein kinase pathway in PC12 cells and regulation by cyclic AMP. *Mol. Cell. Biol.* 14: 6522–6530, 1994.
344. VAN AELST, L., M. BARR, S. MARCUS, A. POLVERINO, AND M. WIGLER. Complex formation between Ras and Raf and other protein kinases. *Proc. Natl. Acad. Sci. USA* 90: 6213–6217, 1993.
345. VAN ANTWERP, D. J., S. J. MARTIN, T. KAFRI, D. R. GREEN, AND I. M. VERMA. Suppression of TNF-α-induced apoptosis by NF-kB. *Science* 274: 787–780, 1996.
346. VAN DER HOORN, F. A., AND J. FIRZLAFF. Complete c-*mos* (rat) nucleotide sequence: presence of conserved domains in c-*mos* proteins. *Nucleic Acids Res.* 12: 2147–2156, 1984.
347. VERLHAC, M. H., H. DE PENNART, B. MARO, M. H. COBB, AND H. J. CLARKE. MAP kinase become stably activated at metaphase and is associated with microtubule-organizing centers during meiotic maturation of mouse oocytes. *Dev. Biol.* 158: 330–340, 1993.
348. VOSSLER, M. R., H. YAO, R. D. YORK, M. G. PAN, C. S. RIM, AND P. J. STORK. cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell* 89: 73–82, 1997.
349. WALKER, J., P. CROWLEY, A. D. MOREMAN, AND J. BARRETT. Biochemical properties of cloned glutathione *S*-transferase from *Schistosoma mansoni* and *Schistosoma japonicum*. *Mol. Biochem. Parasitol.* 61: 255–264, 1993.
350. WAN, Y., K. BENCE, A. HATA, T. KUROSAKI, A. VEILLETTE, AND X.-Y. HUANG. Genetic evidence for a tyrosine kinase cascade preceding the mitogen-activated protein kinase cascade in vertebrate G protein signaling. *J. Biol. Chem.* 272: 17209–17215, 1997.
351. WAN, Y., T. KUROSAKI, AND X.-Y. HUANG. Tyrosine kinases in activation of the MAP kinase cascade by G-protein-coupled receptors. *Nature* 380: 541–544, 1996.
352. WANG, C.-Y., M. W. MAYO, AND A. S. J. BALDWIN. TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kB. *Science* 274: 784–787, 1997.
353. WANG, L. M., A. D. KEEGAN, W. E. PAUL, M. A. HEIDARAN, J. S. GUTKIND, AND J. H. PIERCE. IL-4 activates a distinct signal transduction cascade from IL-3 in factor-dependent myeloid cells. *EMBO J.* 11: 4899–4908, 1992.
354. WANG, W., G. ZHOU, M. C. T. HU, Z. YAO, AND T. H. TAN. Activation of the hematopoietic progenitor kinase-1 (HPK1)-dependent, stress-activated c-Jun N-terminal kinase (JNK) pathway by transforming growth factor beta (TGF-beta)-activated kinase (TAK1), a kinase mediator of TGF beta signal transduction. *J. Biol. Chem.* 272: 22771–22775, 1997.
355. WANG, X., AND D. RON. Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP kinase. *Science* 272: 1347–1349, 1996.
356. WANG, X. M., Y. ZHAI, AND J. E. J. FERRELL. A role for mitogen-activated protein kinase in the spindle assembly checkpoint in XTC cells. *J. Cell Biol.* 137: 433–443, 1997.
357. WANG, X. S., K. DIENER, D. JANNUZZI, D. TROLLINGER, T.-H. TAN, H. LICHENSTEIN, M. ZUKOWSKI, AND Z. YAO. Molecular cloning and characterization of a novel protein kinase with a catalytic domain homologous to mitogen-activated protein kinase kinase kinase. *J. Biol. Chem.* 271: 31607–31611, 1996.
358. WANG, X. S., K. DIENER, C. L. MANTHEY, S. WANG, B. ROSENZWEIG, J. BRAY, J. DELANEY, C. N. COLE, P. Y. CHAN-HUI, N. MANTLO, H. S. LICHENSTEIN, M. ZUKOWSKI, AND Z. YAO. Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. *J. Biol. Chem.* 272: 23668–23674, 1997.
359. WANG, Y., H. P. XU, M. RIGGS, L. RODGERS, AND M. WIGLER. byr2, a *Schizosaccharomyces pombe* gene encoding a protein kinase capable of partial suppression of the ras1 mutant phenotype. *Mol. Cell. Biol.* 11: 3554–3563, 1991.
360. WARBRICK, E., AND P. A. FANTES. The wis1 protein kinase is a dosage-dependent regulator of mitosis in *Schizosaccharomyces pombe*. *EMBO J.* 10: 4291–4299, 1991.
361. WARD, S. G., R. V. PARRY, J. MATTHEWS, AND L. O'NEILL. A p38 MAP kinase inhibitor SB203580 inhibits CD28-dependent T cell proliferation and IL-2 production. *Biochem. Soc. Trans.* 25: 304S, 1997.
362. WASSMANN, K., AND G. AMMERER. Overexpression of the G_1-cyclin gene *CLN2* represses the mating pathway in *Saccharomyces cerevisiae* at the level of the MEKK Ste11. *J. Biol. Chem.* 272: 13180–13188, 1997.
363. WATSON, R., M. K. OSKARSSON, AND G. F. VANDE WOUDE. Human DNA sequence homologous to the transforming gene (*mos*) of Moloney murine sarcoma virus. *Proc. Natl. Acad. Sci. USA* 79: 4078–4082, 1982.
364. WHITEWAY, M., D. DIGNARD, AND D. Y. THOMAS. Dominant nega-

tive selection of heterologous genes: isolation of *Candida albicans* genes that interfere with *Saccharomyces cerevisiae* mating factor-induced cell cycle arrest. *Proc. Natl. Acad. Sci. USA* 89: 9410–9414, 1992.
365. WHITEWAY, M. S., C. WU, T. LEEUW, K. CLARK, A. FOURESTLIEUVIN, D. Y. THOMAS, AND E. LEBERER. Association of the yeast pheromone response G protein beta gamma subunits with the MAP kinase scaffold Ste5p. *Science* 269: 1572–1575, 1995.
366. WHITMARSH, A. J., AND R. J. DAVIS. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *J. Mol. Med.* 74: 589–607, 1996.
367. WIDMANN, C., P. GERWINS, N. LASSIGNAL JOHNSON, M. B. JARPE, AND G. L. JOHNSON. MEKK1, a substrate for DEVD-directed caspases, is involved in genotoxin-induced apoptosis. *Mol. Cell. Biol.* 18: 2416–2429, 1998.
368. WIDMANN, C., S. GIBSON, AND G. L. JOHNSON. Caspase-dependent cleavage of signaling proteins during apoptosis. A turn-off mechanism for anti-apoptotic signals. *J. Biol. Chem.* 273: 7141–7147, 1998.
369. WIDMANN, C., N. LASSIGNAL JOHNSON, A. M. GARDNER, R. J. SMITH, AND G. L. JOHNSON. Potentiation of apoptosis by low dose stress stimuli in cells expressing activated MEK kinase 1. *Oncogene* 15: 2439–2447, 1997.
370. WIESE, M. A mitogen-activated protein (MAP) kinase homologue of *Leishmania mexicana* is essential for parasite survival in the infected host. *EMBO J.* 17: 2619–2628, 1998.
371. WILSON, C., R. ANGLMAYER, O. VICENTE, AND E. HEBERLE-BORS. Molecular cloning, functional expression in *Escherichia coli*, and characterization of multiple mitogen-activated-protein kinases from tobacco. *Eur. J. Biochem.* 233: 249–257, 1995.
372. WILSON, C., N. ELLER, A. GARTNER, O. VICENTE, AND E. HEBERLE-BORS. Isolation and characterization of a tobacco cDNA clone encoding a putative MAP kinase. *Plant Mol. Biol.* 23: 543–551, 1993.
373. WILSON, R., R. AINSCOUGH, K. ANDERSON, C. BAYNES, M. BERKS, J. BONFIELD, J. BURTON, M. CONNELL, J. COOPER, A. COULSON, M. CRAXTON, S. DEAR, Z. DU, R. DURBIN, A. FAVELLO, L. FULTON, A. GARDNER, P. GREEN, T. HAWKINS, L. HILLIER, M. JIER, L. JOHNSTON, M. JONES, J. KERSHAW, J. KIRSTEN, N. LAISTER, P. LATREILLE, J. LIGHTNING, C. LLOYD, A. McMURRAY, B. MORTIMORE, M. O'CALLAGHAN, J. PARSONS, C. PERCY, L. RIFKEN, A. ROOPRA, D. SAUNDERS, R. SHOWNKEEN, N. SMALDON, A. SMITH, E. SONNHAMMER, R. STADEN, J. SULSTON, J. THIERRY-MIEG, K. THOMAS, M. VAUDIN, K. VAUGHAN, R. WATERSTON, A. WATSON, L. WEINSTOCK, J. WILKINSON-SPROAT, AND P. WOHLDMAN. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature* 368: 32–38, 1994.
374. WINSTON, B. W., C. A. LANGE-CARTER, A. M. GARDNER, G. L. JOHNSON, AND D. W. H. RICHES. Tumor necrosis factor α rapidly activates the mitogen-activated protein kinase (MAPK) cascade in a MAPK kinase kinase-dependent, c-Raf-1-independent fashion in mouse macrophages. *Proc. Natl. Acad. Sci. USA* 92: 1614–1618, 1995.
375. WINSTON, L. A., AND T. HUNTER. Intracellular signalling: putting JAKs on the kinase MAP. *Curr. Biol.* 6: 668–671, 1996.
376. WOJNOWSKI, L., A. M. ZIMMER, T. W. BECK, H. HAHN, R. BERNAL, U. R. RAPP, AND A. ZIMMER. Endothelial apoptosis in Braf-deficient mice. *Nature Genet.* 16: 293–297, 1997.
377. WU, J., J. K. HARRISON, P. DENT, K. R. LYNCH, M. J. WEBER, AND T. W. STURGILL. Identification and characterization of a new mammalian mitogen-activated protein kinase kinase, MKK2. *Mol. Cell. Biol.* 13: 4539–4548, 1993.
378. WU, J., J. K. HARRISON, L. A. VINCENT, C. HAYSTEAD, T. A. HAYSTEAD, H. MICHEL, D. F. HUNT, K. R. LYNCH, AND T. W. STURGILL. Molecular structure of a protein-tyrosine/threonine kinase activating p42 mitogen-acitivated protein (MAP) kinase: MAP kinase kinase. *Proc. Natl. Acad. Sci. USA* 90: 173–177, 1993.
379. WU, X., S. J. NOH, G. ZHOU, J. E. DIXON, AND K.-L. GUAN. Selective activation of MEK1 but not MEK2 by A-Raf from epidermal growth factor-stimulated HeLa cells. *J. Biol. Chem.* 271: 3265–3271, 1996.
380. WU, Y., AND M. HAN. Suppression of activated Let-60 ras protein defines a role of *Caenorhabditis elegans* Sur-1 MAP kinase in vulval differentiation. *Genes Dev.* 8: 147–159, 1994.
381. WU, Y., M. HAN, AND K. L. GUAN. MEK-2, a *Caenorhabditis elegans* MAP kinase kinase, functions in Ras-mediated vulval induction and other developmental events. *Genes Dev.* 9: 742–755, 1995.
382. WU, Z., J. WU, E. JACINTO, AND M. KARIN. Molecular cloning and characterization of human JNKK2, a novel Jun NH_2-terminal kinase-specific kinase. *Mol. Cell. Biol.* 17: 7407–7416, 1997.
383. XIA, Z., M. DICKENS, J. RAINGEAUD, R. J. DAVIS, AND M. E. GREENBERG. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 270: 1326–1331, 1995.
384. XU, H. P., M. WHITE, S. MARCUS, AND M. WIGLER. Concerted action of Ras and G proteins in the sexual response pathways of *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* 14: 50–58, 1994.
385. XU, S., D. J. ROBBINS, L. B. CHRISTERSON, J. M. ENGLISH, C. A. VANDERBILT, AND M. H. COBB. Cloning of rat MEK kinase 1 cDNA reveals an endogenous membrane-associated 195-kDa protein with a large regulatory domain. *Proc. Natl. Acad. Sci. USA* 93: 5291–5295, 1996.
386. YAFFE, M. B., K. RITTINGER, S. VOLINIA, P. R. CARON, A. AITKEN, H. LEFFERS, S. J. GAMBLIN, S. J. SMERDON, AND L. C. CANTLEY. The structural basis for 14-3-3: phosphopeptide binding specificity. *Cell* 91: 961–971, 1997.
387. YAMAGUCHI, K., K. SHIRAKABE, H. SHIBUYA, K. IRIE, I. OISHI, N. UENO, T. TANIGUCHI, E. NISHIDA, AND K. MATSUMOTO. Identification of a member of the MAPKKK family as a potential mediator of TGF-β signal transduction. *Science* 270: 2008–2011, 1995.
388. YAMAMORI, B., S. KURODA, K. SHIMIZU, K. FUKUI, T. TAKAI, AND Y. OHTSUKA. Purification of a Ras-dependent mitogen-activated protein kinase kinase kinase from bovine brain cytosol and its identification as a complex of B-Raf and 14-3-3 proteins. *J. Biol. Chem.* 270: 11723–11726, 1995.
389. YAMAUCHI, J., M. NAGAO, Y. KAZIRO, AND H. ITOH. Activation of p38 mitogen-activated protein kinase by signaling through G protein-coupled receptors. Involvment of $G\beta\gamma$ and $G\alpha_{q/11}$ subunits. *J. Biol. Chem.* 272: 27771–27777, 1997.
390. YANG, D. D., C. Y. KUAN, A. J. WHITMARSH, M. RINCON, T. S. ZHENG, R. J. DAVIS, P. RAKIC, AND R. A. FLAVELL. Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* 389: 865–870, 1997.
391. YANG, X., R. KHOSRAVI-FAR, H. Y. CHANG, AND D. BALTIMORE. Daax, a novel Fas-binding protein that activates JNK and apoptosis. *Cell* 89: 1067–1076, 1997.
392. YASHAR, B. M., C. KELLEY, K. YEE, B. ERREDE, AND L. I. ZON. Novel members of the mitogen-activated protein kinase activator family in *Xenopus laevis*. *Mol. Cell. Biol.* 13: 5738–5748, 1993.
393. YIN, M.-J., L. B. CHRISTERSON, Y. YAMAMOTO, Y.-T. KWAK, S. XU, F. MERCURIO, M. BARBOSA, M. H. COBB, AND R. B. GAYNOR. HTLV-1 Tax protein binds to MEKK1 to stimulate IkB kinase activity and NF-kB activation. *Cell* 93: 875–884, 1998.
394. ZAHEER, A., AND R. LIM. In vitro inhibition of MAP kinase (ERK1/ERK2) activity by phosphorylated glia maturation factor (GMF). *Biochemistry* 35: 6283–6288, 1996.
395. ZAITSEVSKAYA-CARTER, T., AND J. A. COOPER. Spm1, a stress-activated MAP kinase that regulates morphogenesis in *S. pombe*. *EMBO J.* 16: 1318–1331, 1997.
396. ZECHNER, D., D. J. THUERAUF, D. S. HANFORD, P. M. McDONOUGH, AND C. C. GLEMBOTSKI. A role for the p38 mitogen-activated protein kinase pathway in myocardial cell growth, sarcomeric organization, and cardiac-specific gene expression. *J. Cell Biol.* 139: 115–127, 1997.
397. ZERVOS, A. S., L. FACCIO, J. P. GATTO, J. M. KYRIAKIS, AND R. BRENT. Mxi2, a mitogen-activated protein kinase that recognizes and phosphorylates Max protein. *Proc. Natl. Acad. Sci. USA* 92: 10531–10534, 1995.
398. ZHANG, S., J. HUAN, M. A. SELLS, J. CHERNOFF, U. G. KNAUS, R. J. ULEVITCH, AND G. M. BOKOCH. Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1. *J. Biol. Chem.* 270: 23934–23936, 1995.
399. ZHANG, S., AND D. F. KLESSIG. Salicyclic acid activates a 48-kilodalton MAP kinase in tobacco. *Plant Cell* 9: 809–824, 1997.
400. ZHANG, X. F., J. SETTLEMAN, J. M. KYRIAKIS, E. TAKEUCHI-SUZUKI, S. J. ELLEDGE, C. J. MARSHALL, J. T. BRUDER, U. R. RAPP, AND J. AVRUCH. Normal and oncogenic p21ras proteins

bind to the amino-terminal regulatory domain of c-Raf-1. *Nature* 364: 308–313, 1993.

401. ZHANG, Y., X. FENG, R. WE, AND R. DERYNCK. Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. *Nature* 383: 168–172, 1996.
402. ZHENG, C.-F., AND K.-L. GUAN. Cloning and characterization of two distinct human extracellular signal-regulated kinase activator kinases, MEK1 and MEK2. *J. Biol. Chem.* 268: 11435–11439, 1993.
404. ZHENG, C. F., M. OHMICHI, A. R. SALTIEL, AND K. L. GUAN. Growth factor induced MEK activation is primarily mediated by an activator different from c-*raf*. *Biochemistry* 33: 5595–5599, 1994.
405. ZHOU, G., Z. Q. BAO, AND J. E. DIXON. Components of a new human protein kinase signal transduction pathway. *J. Biol. Chem.* 270: 12665–12669, 1995.
406. ZHU, A. X., Y. ZHAO, D. E. MOLLER, AND J. S. FLIER. Cloning and characterization of p97MAPK, a novel human homolog of rat ERK-3. *Mol. Cell. Biol.* 14: 8202–8211, 1994.
407. ZHU, X., AND R. K. ASSOIAN. Integrin-dependent activation of MAP kinase: a link to shape-dependent cell proliferation. *Mol. Biol. Cell* 6: 273–282, 1995.
408. ZIMMERMANN, S., C. ROMMEL, A. ZIOGAS, J. LOVRIC, K. MOELLING, AND G. RADZIWILL. MEK1 mediates a positive feedback on Raf-1 activity independently of Ras and src. *Oncogene* 15: 1503–1511, 1997.
409. ZINCK, R., M. A. CAHILL, M. KRACHT, C. SACHSENMAIER, R. A. HIPSKIND, AND A. NORDHEIM. Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. *Mol. Cell. Biol.* 15: 4930–4938, 1995.